# Longitudinal monitoring of parasites in individual wild primates

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To Teemu,

and 139 other mouse lemurs who made this possible.

Yö tuntee kaiken sen mikä on,
mut mitä ei nää:
pelot ja painajaiset,
ja myös sun naurujes syyn.
Ja jos sä horjahtelet,
se ottaa sut syleilyyn.
Ja kuule, se kuiskii hei tuu,
se kutsuu sua seikkailuun.

Maija Vilkkumaa
*Yöllä*

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# List of original publications

**I** Tuomas Aivelo, Alan Medlar, Ari Löytynoja, Juha Laakkonen, Jukka Jernvall. High-throughput barcoding of fecal samples identifies the composition of parasitic nematode communities. Manuscript.

**II** Alan Medlar, Tuomas Aivelo, Ari Löytynoja. (2014). Séance: reference-based phylogenetic analysis for 18S rRNA studies. B*MC Evolutionary Biology* **14**,235.
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**III** Tuomas Aivelo, Alan Medlar, Ari Löytynoja, Juha Laakkonen, Jukka Jernvall. (2015). Tracking year-to-year changes in intestinal nematode communities of rufous mouse lemurs (*Microcebus rufus*). *Parasitology*, **142**,1095-1197.
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# Contributions of authors

In study I, the original idea and the study design were conceived by TA, JL and JJ. Data collection in the field and laboratory work was performed by TA. Bioinformatic analysis was done by AM and AL and the data analysis by TA and AM. The manuscript was prepared by TA and AM with comments from other authors.
In study II, AM and AL contributed to software design and implementation. Data generated by TA. AM, TA, AL wrote the manuscript. All authors read and approved the final manuscript.
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# Glossary

**Component community** All the individuals of several parasite species at the same developmental stage, e.g., all adult helminths in mouse lemurs' intestines

**Compound community** The union of all the different parasite communities, e.g., all adult helminths in mouse lemurs' intestines and their larval forms in their intermediate hosts

**Cryptic species** Species which cannot be distinguished by morphological differences

**Definitive host** The host where the sexual reproduction of parasite takes place

**Endemic species** Species which are native to a given geographical area

**Exotic species** Species which have been introduced to a new area in which they are not endemic. Exotic species can be invasive.

**Host range** All of the hosts where a certain parasite species is able to exist and reproduce

**Infracommunity** The parasite community in a single host individual, i.e, all adult helminths in individual mouse lemur's intestine. Infracommunities are the building blocks of the component communities

**Intermediate host** A host where parasite reproduces asexually

**Invasive species** Non-endemic species which can outcompete endemic species and thus cause changes in community structure.

**Macro- and microparasite** The difference between micro- and macroparasites is not clear-cut and thus more one of convenience. In general, parasitic viruses, bacteria and protists are considered microparasites while animal parasites are macroparasites. Macroparasites tend to have different epidemiological properties as microparasites: in comparison to the microparasites, they are long-lived, large-bodied and slowly reproducing. Thus it makes sense to track individual parasites, rather than infected hosts like with microparasites

**Next-generation sequencing** Sequencing methods which result to several sequences from one run, not only one consensus sequence

**Parasite load** The total number of parasite individuals in a host individual

**Pathogenicity** The ability of a pathogen to cause damage to the host. Related, and sometimes interchangeable concept, with virulence

**Prevalence** The proportion of host population which has a given parasite

**Resistance** The host's ability to limit parasite load during infection.

**Tolerance** The host's ability to limit parasite infection's effects on health and fitness.

**Virulence** The measure of the fitness effects of pathogens on their host. While there is a wide spectrum of definitions, normally the virulence is defined as a property of the parasite measured in relation to the host.

# Abstract

Parasite community dynamics is one of the central themes in contemporary parasitology. While between-host dynamics has been studied for a long time, within-host dynamics is less well studied. My aim was to identify which factors affect the parasite community during the lifetime of individual hosts by following longitudinally several individuals from a long-living species. Specifically, I was interested in how the dynamics of infra- and component communities differ from one another and which traits explain the variation in infracommunities.

I studied rufous mouse lemur (*Microcebus rufus*), which is a primate living in the eastern montane rainforests of Madagascar. Mouse lemur is a well-suited study species as it can live for up to ten years in the wild. Due to its small size, the population density is high and trapping straightforward. Nematodes are the most common helminths found in mouse lemurs, but their identification is difficult. Typically, the nematodes are identified from adult specimens, but for longitudinal studies, this is not possible, as we cannot dissect the host individuals. In addition, morphological differences between species are small and we expected to encounter previously undescribed species. These difficulties led to the development of a new method, based on metabarcoding, to identify parasitic nematodes from fecal samples. The method I developed encompasses all steps from fieldwork to sequence analysis.

Despite numerous confounding factors, the method managed to amplify and analyze half of the samples collected. Whilst there is room for further improvements, the main advantage is that the method works well for different host species, for example mouse lemurs and gastropods. In principle, this method works for all species of nematode, including free-living soil nematodes. Nevertheless, the resolution of identification do not allow for species-level identification.

The variation in the parasite community inside individual hosts was extensive, but at the population level remained stable. Most of the parasites belonged to the putative species thought to be Strongyloides sp. The reason for this species success might be its lifecycle, the parasite can live in the intestine or as a free-living form in the soil.

Due to the limited number of samples, the factors affecting the dynamics in individual mouse lemurs are difficult to analyze. It appears that sex and age do not have an effect on either parasite load or variation in parasite dynamics. Nevertheless, body condition appears to bear a consequence with the individuals in better condition having more parasite species in addition to higher fecal egg loads. The reason may be that those individuals are able to sustain larger populations of parasites, or that they are more tolerant to parasites. Hibernation could lead to the extinction of the nematode community, whereas higher precipitation appeared to lead to higher prevalences.

This work gives insights into the dynamics of parasite communities both at the host inforthe need for longitudinal studies as parasite community dynamics in host individual-level cannot be inferred from the host population-level The method I have developed can be used to perform more efficient and faster surveys of previously unknown parasite communities, though further development is necessary for better reliability.

# Tiivistelmä

Loisyhteisöjen muuttuminen ajan mittaan on yksi keskeisiä loistutkimuksen huomion kohteita. Loisten esiintymistä isäntäpopulaatiossa on seurattu pitkään. Sen sijaan tunnetaan huonosti, miten yksittäisen isäntäyksilön loislajisto muuttuu yksilön elinkaaren aikana. Tarkoitukseni olikin selvittää, kuinka paljon loislajisto muuttuu yhden isäntäyksilön elämän aikana seuraamalla loislajistoa useissa pitkäikäisissä yksilöissä.

Tutkin ruskohiirimakia (*Microcebus rufus*), joka on Madagaskarin itäosan vuoristosademetsissä asuva kädellinen. Kokoon nähden pitkä ikä tekee hiirimakista oivallisen tutkimuskohteen: hiirimakit voivat elää vapaana jopa kymmenen vuoden ikäisiksi. Pienen koon ansiosta hiirimakeja elää tiheässä ja näytteiden kerääminen on helppoa.

Sukkulamadot olivat yleisin hiirimakien suolistoloinen. Tyypillisesti suolistoloiset tunnistetaan aikuisista yksilöistä, mutta tämä ei ollut mahdollista pitkittäisseurannassa, sillä emme pääse käsiksi suolistossa asuviin matoihin. Lisäksi sukkulamatojen tunnistaminen on vaikeaa, sillä sukkulamatolajien väliset erot ovat pieniä ja tutkimiamme lajeja ei ole todennäköisesti aiemmin kuvattu. Kehitin uuden, geneettisen tunnistukseen perustuvan menetelmän loislajiston tunnistamiseksi ulostenäytteistä löytyvistä munista ja toukista. Kehittämäni menetelmä kattaa kaikki vaiheet kenttätyöstä loissekvenssien käsittelyyn.

Menetelmä toimi suhteellisen hyvin: noin puolet näytteistä onnistuttiin analysoimaan. Menetelmä on lupaava, mutta vaatii jatkokehittelyä. Erityinen tunnistusmenetelmän etu on, että sama menetelmä toimi hyvin erilaisille isäntälajeille, kuten hiirimakeille, rotille, sammakoille ja kotiloille. Periaatteessa menetelmä voi toimia myös esimerkiksi maaperän sukkulamadoille.

Tutkimukseni tulokset paljastivat lisäksi, että vaihtelu loislajistossa yhden yksilön sisällä oli suurta. Tästä huolimatta isäntäpopulaation tasolla loislajisto pysyi hyvin vakaana. Suurin osa loisista kuului yhteen lajiin, joka kuuluu todennäköisesti Strongyloides –sukuun. Syynä tämän lajin menestykseen saattaa olla loisen elinkierto: loinen voi elää paitsi hiirimakien suolistossa, myös vapaana toukkamuotona maaperässä. Aineiston pienuuden takia yksittäisen hiirimakin loislajistoon vaikuttavista tekijöistä on vaikea tehdä johtopäätöksiä: vaikuttaa siltä että sukupuolella tai iällä ei ole merkitystä loisten määrään tai vaihteluun. Sen sijaan yksilöiden kunnolla on merkitystä: paremmassa kunnossa olevilla yksilöillä on enemmän loislajeja ja loiset munivat enemmän. Syynä tähän voi olla joko se että paremmassa kunnossa olevat yksilöt voivat elättää suurempia loismääriä tai sitten se, että paremmassa kunnossa olevat yksilöt sietävät paremmin loisia. Horrostaminen saattaa johtaa sukkulamatoyhteisöjen tuhoon, kun taas suuremmat sademäärät lisäävät loisten määriä.

Kokonaisuutena tutkimukseni avaa uusia näkökulmia loislajiston muutokseen sekä yksittäisissä isäntäyksilöissä että isäntäpopulaatiossa. Erityisesti tutkimukseni alleviivaa pitkittäistutkimusten tärkeyttä: isäntäpopulaatiotason dynamiikasta ei pystytä päättelemään miten loisdynamiikka toimii yhden isäntäyksilön kohdalla. Kehittämäni menetelmällä voi suhteellisen helposti tunnistaa aiempaa nopeammin ja tehokkaammin aiemmin tuntemattoman suoliston sukkulamatojen yhteisön.

# Famintinana

Ny fahavitrihan'ny vondrona Katsentsitra dia anisan'ireo lohahevitra lehibe eo amin sehatrin'ny fikaroahana amin'izao fotoana izao. Na dia efa voafaritra nandritra ny fotoana ela aza ny dinamika eo amin'ireo samy itobian'ny Katsentsitra, ny fivoarana ao anatin'ny toby iray kosa dia mbola tsy ampy. Ny tanjoko eto dia ny hamakafaka ireo antony mahatonga ny vondrona Katsentsitra ho ao amin'ny biby iray mandritra ny androm-piainany, amin'ny alalan'ny fanarahamaso mitandavana ireo karazam-biby velona .

Nianatra ny gidro Tsidy ala aho, izay karazana "primate" misy any amin'ny tandavan'ala mando antsinanan'i Madagascar. Ny Tsidy ala dia karazam-biby azo hianarana tsara noho izy afaka velona mihoatra ny folo taona anaty ala. Noho ny haben'izy ireo kely, dia ambony ny hakitroky ny mponina ary mora samborina izy ireo.

Ny "nematodes" dia karazana kankana misy ao amin'ny Tsidy ala, kanefa sarotra ny manavaka azy ireo. Azo atao ihany anefa ny manavaka azy ireo amin'ny alalan'ireo izay efa lehibe, kanefa noho ny fanadihadiana mitandavana dia mety tsy ho azo tanterahana io. Ny fahasamihafan'ny bikan'ireo karazany dia kely ary mety mbola hahazo karazany hafa efa azo ihany izahay. Noho izany, namolavola fomba vaovao izaho, izay miankina amin'ny fanavahana ara-pototarazo ireo "nematode" anaty taim-biby. Ny fomba izay nentiko namolavolaina dia niainga avy amin'ireo asa avy any anaty ala hatramin'ny fikirakirana ny filaharan'ny fototarazo.

Nandaitra ny fomba nentina niasa: Ny antsasaky ny santionan-javatra dia voavolavola sy voakirakira daholo, ny fomba nampiasaina dia mahavelom-panantenana saingy kosa mila fanatsarana amin'ny ho avy. Ny tombony manokana dia mety daholo ny fomba nentina tamin'ireo karazana biby fitobiana, ohatra eo amin'ny Tsidy ala sy ny Sifotra. .

Ny fahasamihafan'ireo vondrona katsentsitra ao anatin'ny biby tsirairay avy dia miovaova be, raha eo amin'ny haavon'ny mponina dia marin-toerana izy ireo. Ny ankamaroan'ireo katsentsitra dia novinavinaina ho ao anatin'ny karazana Strongyloides sp. Ny hevitra nahatonga io dia noho ny fiainan'izy ireo: afaka mivelona anaty tsinay ary koa anaty tany.

Noho ny santionan-javatra voafetra, dia sarotra nofakafakaina ny fahavitrihan'ny Katsentsitra tao amin'ireo Tsidy ala tsirairay. Ny taona na ny fananahana dia ohatry ny tsy misy nifandraisany tamin'ny fananana Katsentsitra na koa ny fiovaovan'ny fahavitrihan'ny Katsentsitra. Kanefa kosa, ny toe-batana dia ohatry ny nampisy fiantraikany: izay rehetra nanana toe-batana tsara dia nahitana Katsentsitra sy atodiny betsaka tao anaty tainy. Ny anton'izany dia mety ho izy ireo afaka nitazona vondrona Katsentsitra maro, na koa nanana toetra afaka nandefitra.

Amin'ny ankapobeny, ity asa ity dia manome endrika isehoan'ny fahavitrihan'ny Katsentsitra eo amin'ny haavon'ny biby tsirairay na koa eo amin'ny vondrom-biby iray. Ny fomba nentiko namolavolana ity asa ity dia azo ampiasaina tsara amin'ireo karazam-pikarohana haingana sy mandaitra ho amin'ireo vondrona Katsentsitra izay mbola tsy fantatra tany aloha tany.

*Translated by Andry Herman Rafalinirina*

# Summary

# 1. Introduction

## 1.1. Parasites are a world-changing force

Probably most species in the world have parasites. It has been estimated that half of the species in the world are parasites (Windsor, 1998; Dobson *et al.*, 2008; Poulin, 2014). These are the two most important reasons to study parasites and their dynamics in a wild host population. Parasites are important from an ecological point of view as their persistence and exploitation of host resources can be the demise of their hosts. This in turn translates into great evolutionary pressure making parasites one of the most important factors shaping evolution. Parasites are, for example, considered to be one of the reasons for the existence of sex (Hamilton, 1980).

Due to their immense number, it is no surprise that parasites are economically important. They plague humans, domestic animals and food crops (Torgerson and Macpherson, 2011; Ferris *et al.*, 2012). Natural resources, such as game or forestry, are affected by parasites. The central position of parasites in any ecosystem makes them also necessary to take into account in conservation biology with the effects of habitat fragmentation and degradation compounded by changes in parasite interactions (Thompson *et al.*, 2010; Nichols and Gómez, 2011). Parasites are not necessarily a negative force as their central role means they are also an important part of biodiversity. Parasites can be used in biological control of herbivores and predators (Gaugler *et al.*, 1997), have their own intrinsic value as a part of biodiversity (Gómez and Nichols, 2013) and their absence in humans has been linked to increased prevalence of autoimmune disorders (Strachan, 1989).

Considering the topics mentioned above, it is imperative that we understand how parasites live, reproduce and affect their hosts. We have to know how they are spread and transmitted in their host populations to understand how they parasites shape ecosystems. Until recently, however, parasite studies primarily looked at systems with one host and one parasite. Parasitology as a field has started to expand to more complex systems with multiple hosts and multiple parasites (Rigaud *et al.*, 2010). The community approach to parasitology is still in its early stages (Fenton *et al.*, 2010; Viney and Graham, 2013). My motivation for this thesis was the distinct lack of studies following parasite community succession in individual hosts. Rather, a majority of studies have focused on the host population level by following changes in parasite communities by surveying large populations of hosts at a single time-point. Examples of successful long-term studies include Soay sheep (Wilson *et al.*, 2004) and voles of Kielder (Turner *et al.*, 2014). The lack of individual host-based studies might be due to practical concerns as it is easier to trap, kill and dissect small mammals to survey intestinal parasites. It is much more difficult to survey the parasite communities in living animals. Longitudinal studies require not only methods to recapture and identify hosts but also non-invasive survey methods for the parasites themselves.

My thesis has two distinct components. I have developed a method which allows us to follow longitudinally intestinal nematodes which are sampled from the feces. I then used this method to follow several individuals for subsequent years to uncover parasite dynamics. In this first part of the introduction I cover the important theoretical questions: how we define a parasite, how parasite communities are formed and why they are difficult to study. As I worked primarily with macroparasites of mouse lemurs, the emphasis will be on intestinal parasites of mammals. I will discuss different molecular methods used for parasite identification, detail my experimental setup and explain the central results.

## 1.2. Parasite distribution and occurrence

To consider how parasite communities within mouse lemurs are shaped, we must ask two crucial questions: i) why are certain parasite species able to infect mouse lemurs? and ii) what factors could have affected the formation of the parasite assemblage in same vicinity?

Some parasite species can infect only one (definitive) host species, whereas others are much more general in their preference. There is a wide range of possibilities to explain this variation. Parasites might have only one intermediate host species but many potential definitive hosts or vice versa. The host range is also continuous with the parasite able to infect a wide group of species (tetrapods, for example) or only close relatives (primates). It is possible that if parasites have many hosts, then these tend to be phylogenetically close relatives (Pedersen *et al.*, 2005; Davies and Pedersen, 2008; Pedersen and Davies, 2009; Huang *et al.*, 2013), but compatibility could also be due to geographical closeness, host environment or immunological similarity (Poulin and Keeney, 2008; Poulin, 2010; Dallas and Presley, 2014; Hoberg and Brooks, 2015). Indeed, mouse lemur parasites are still poorly known, but evidence suggests that many species of parasite are shared with other lemurs (Irwin, 2008) and for example, invasive black rats (Rasambainarivo *et al.*, 2013). The extent of species-specific parasites in mouse lemur species is unknown, due to the lack of taxonomical studies on their parasites.

It should be stated that if a particular parasite is not present in a given host species, it does not mean that it cannot infect those hosts. It could simply be the case that parasite and host do not encounter one another in nature. Thus host range is also a question of compatibility for the host species life cycle (Table 1; Schmid-Hempel, 2011). We know that most parasites are highly plastic in their responses to the environment (Agosta and Klemens, 2008) as there are wide variations in body size (Szalai and Dick, 1989), infection route (Vizoso and Ebert, 2005), fecundity (Loot *et al.*, 2008) and in life span (Gardner *et al.*, 2006). Indeed, the colonization of new host species do not necessarily require evolutionary adaptation (Hoberg and Brooks, 2015). Here parasitology meets general ecology: while it is debated how important local adaptation is (Hendry and Gonzales, 2008), it is also an open question how well parasites and hosts are locally adapted (Lajeunesse and Forbes, 2002; Greischar and Koskella, 2007; Schulte *et al.*, 2011)

To understand the local parasite assemblage, it is crucial to consider parasite biogeography: how the parasite community available in any given area has formed through historical processes guiding both evolution and the spread of parasite species. Different geographical areas have different histories that need to be taken into account when considering the composition of the parasite community (Hoberg *et al.*, 2012).

There is considerable variation in parasite species number depending on the species. In a given host species, the host density and host geographical range are the two best predictors of parasite richness (Arneberg *et al.*, 1998; Kamiya *et al.*, 2014; Morand, 2015). Larger sized hosts can harbor more parasite species (Morand and Poulin, 1998) for several reasons: large-bodied animals have longer life spans and provide more niches and resources for parasites (Cardon *et al.*, 2011). The existence of niches appears to be crucial as there is, at best, a weak relationship between longevity of species and parasite richness (Gregory *et al.*, 1996; Morand and Harvey, 2000; Cooper *et al.*, 2012). Wider geographical range results in encounter with more parasite species and larger overlap with other potential host species (Guégan and Kennedy, 1993; Nunn and Dokey, 2006). While population density is an important factor for parasite richness (Nunn *et al.*, 2003; Nunn and Dokey, 2006; Kamiya *et al.*, 2014), the link between host group size and parasite richness is not straightforward: species with larger group sizes also have higher community modularity, which in turn decreases parasites success (Griffin and Nunn, 2011; Cardon *et al.*, 2011; Patterson and Ruckstuhl, 2013). There is

## Box 1: What is a parasite?

As seems to be true for any biological concept, defining a parasite is notoriously difficult. Systematics is of limited assistance as, for example, metazoan parasites are divided in at least 12 different phyla (Poulin and Morand, 2004). The three most species-rich parasite taxons are roundworms (Nematoda), flatworms (Platyhelminthes) and arthropods, all of which also contain thousands of non-parasitic species (Bush *et al.*, 2001).

In general, parasitism is a symbiotic relationship between species, where the parasite benefits at the expense of the host. This is far from a clear-cut definition. I have presented a suggestion for the definition in the two-dimensional graph (Fig. 1) that has the fitness effect on the y-axis and the closeness of interaction on the x-axis. Symbiosis will be used in this thesis as a term for any close interspecific relation, be it parasitic, commensalistic or mutualistic. The fitness component of parasitism is a continuum from mutualistic relationships where both individuals benefit, through commensalism, where one species benefits, but not at the expense of the other, to parasitic relationships that disadvantage the host (Leung and Poulin, 2008). The closeness component is a continuum from predation to the full-fledged parasitism. Parasitoids are parasite-like species that lay their eggs in host individuals; the resultant larva then eats the host from the inside out. When the adult parasitoid emerges from the host, the host is killed (Reuter, 1913). The main distinction between parasites and parasitoids is that parasitoids act, from an ecological modelling point of view more like predators than parasites (Godfray, 1994).

As Leung and Poulin (2008) have pointed out the classical definitions of parasitism do not reflect the dynamics of parasitism. In fact, it might be reasonable to add a third axis to the figure: the life cycle of the parasite. There can be temporal variation both in the level of symbionticism and in the fitness costs of interspecific interactions. For example, some parasitic species are only parasites during certain parts of their life cycle: many nematodes have a free-living form in water or soil, while they are in search of their next host. Many parasites infected through a fecal-oral route have forms that can survive for extended periods outside of host animals (Pietrock and Marcogliese, 2003).

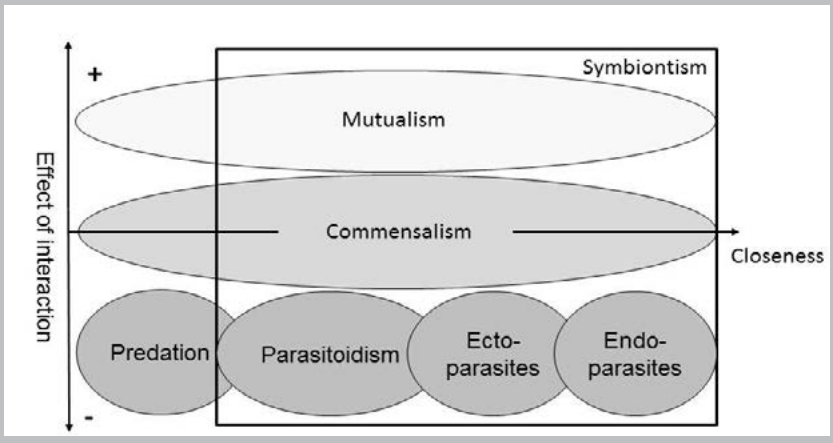


**Figure 1**: The definition of parasitism presented as a two-dimensional graph. Parasitism has a negative effect on the other participant (host) and includes close relationships between participants (symbiontism). The closeness can be understood both in evolutionary terms or physical terms. The graph is based on Bruno Betschart's lectures in University of Neuchâtel (2007).

debate on what is are more important for primate parasite diversity, host individual-level drivers or population-level drivers (Benavides *et al.*, 2012).

Invasions of non-endemic fauna are a central force in the biogeography of parasites. It seems most of the invasion processes are case-dependent and complex, and it is difficult to outline the general properties of invasions (Hoberg, 2010). In general, anthropogenic invasions occur across long distances in short timescales, while natural and climatic invasions are much slower. All invasions lead to mosaic faunal assemblage, with human-mediated invasions leading to more fragmented parasite assemblages (Hoberg and Brooks, 2008).

There are some generalities in outcomes of parasite invasions, however, it appears that many invasive host species get rid of many parasite species after moving on to another geographical location (Torchin *et al.*, 2003). Invasive parasites appear to have better chances of establishing themselves in small or distant islands (Wikelski *et al.*, 2004). Abundant and vertically transmitted parasites also seem to survive better (Prenter *et al.*, 2004). In Madagascar, there is evidence of an influx of parasites introduced by invasive Rattus rattus (Joyeux and Baer, 1936; Sandosham 1950; Quentin and Durette-Desset, 1974; Durette-Desset *et al.*, 2002). Nevertheless, the outcomes of invasions are highly variable. Some invasive parasites might thrive in endemic species much better than their original invasive species hosts (Hatcher *et al.*, 2012), leading to potential changes in host assemblage. Invasive host species also acquire new parasite species from endemic hosts, but this proportion seems to be much smaller than the invasive species acquired by endemic hosts (Marr *et al.*, 2007). The invasive host species can also provide a dilution effect – they take most of the parasite load whereas the endemic species have a reduced parasite load (Telfer *et al.*, 2005; Johnson and Thieltges, 2010).

There is recent interest in parasite introductions to Madagascar, due to the growing threat from anthroponoses and zoonoses (Rasambainarivo *et al.*, 2013, Yanagida *et al.*, 2014) and to assess the effect of black rat invasion on human and animal pathogens (Laakkonen *et al.*, 2003; Vogler *et al.*, 2011). Nevertheless, the prehistorical parasite invasion history to Madagascar is rarely studied. We know that *Plasmodium* and Makialgales mites were present in lemurs pre-colonization (Pacheco *et al.*, 2011, Bochkov *et al.*, 2011) and *Hymenolepis* radiation concurs with colonization history (Haukisalmi, *pers.comm.*). It seems likely that mouse lemur parasites are divided into three groups: those present in ancestral species before colonization (e.g., *Plasmodium*), those from host switches from endemic species within Madagascar (e.g. *Hymenolepis*) and those introduced by humans or their companions (*Giardia* and *Cryptosporidium*?).

## 1.3. Sampling parasite community within single host

During my research I surveyed several parasite species simultaneously, meaning I investigated a parasite community - that is a coexisting group of parasite populations (Bush *et al.*, 1997). The parasite population is composed of all the individuals of a given species that live in the same area. The intestine of a small mammal contains a parasite community, which is composed of several interacting parasite populations.

Parasites can have direct or indirect life cycles. Parasites with direct life cycles have only one host, whereas an indirect lifecycle means that the parasite has one or more intermediate hosts and one definitive host (Poulin and Morand, 2004). Thus, parasites with direct lifecycles can therefore be easily surveyed by studying all of the infected host individuals. In contrast, parasites with indirect life cycles have different discrete developmental stages in different host species, i.e., different host populations. In my study, I expect some of the mouse lemur parasites to have insects as their intermediate hosts as mouse lemurs are omnivores which also consume significant amounts of insects.

It would be exceptionally difficult, if not im-

possible, to sample each parasite species in all of the communities in which it takes part. For example, the parasites which have poorly understood indirect life cycles, which might have free-living forms or which have several host species are practically impossible to be surveyed in every community. Therefore, my sampling is limited to the mammalian, anuran and gastropod hosts of nematodes. Thus, depending on the species, I might sample all component communities or just a small subset of them.

### 1.3.1. Parasite population of single species within host

Macroparasites are normally aggregated in the host population, i.e., some host individuals have much more parasite individuals than a majority of other hosts (Woolhouse *et al.*, 1997; Perkins *et al.*, 2003). This is often depicted in the "long-tail" of parasite loads (Fig. 2).The aggregation being so great that the average number of parasites per host is a poor descriptor of infection level (Rózsa *et al.*, 2000), implying that variance needs to be taken into account as well. There are parasite and host traits that decrease aggregation of parasite individuals (of a single species). Intensity-dependent parasite mortality (i.e., parasites in highly infected hosts are more likely to die), parasite-induced host mortality (i.e., hosts with high parasite loads are more likely to die) and high prevalence of parasites have all been shown to decrease aggregation (Woolhouse *et al.*, 1997). Spatial aggregation of resources which are relevant to parasite transmission (intermediate hosts used as food, nesting sites, watering holes) can lead to higher aggregation (Shaw and Dobson, 1995). There is low heritability of nematode burden (Beraldi *et al.*, 2007) and the candidate gene approach by Brown *et al.* (2013) did not discover any significant genetic associations to this trait. Thus there does not seem to be a genetic basis in host individuals for parasite individual aggregation. Indeed, aggregation can be caused by random variation in host individuals (Gourbière *et al.*, 2015).

Concerning the host, there is at least two clear traits that explain individual differences in parasite load: host condition and host sex. A host's physical condition can affect the parasite community in many ways: parasites get their nutrients directly from the host, so the better condition the host is in, the more nutrients there are available for parasites (Råberg, 2014). Host condition is also fundamental for the efficacy of the immune system; therefore, a better physical condition can lead to a more potent immune defense against parasites (Sheldon and Verhulst, 1996; Ujvari and Madsen, 2005). However, the immune defense is costly and there is a trade-off between immune defense and other energetically costly life history characteristics such as mating (Schmid-Hempel, 2003; Fedorka *et al.*, 2004), gestation (Lee, 2006; Hawley and Altizer, 2011), growth (Soler *et al.*, 2003) etc. While resistance has been seen as the most important host trait in the regulation of parasites, it does not explain all phenomena, for example, there is a wide variation in how strong the negative effects of higher parasite load is for hosts (Råberg *et al.*, 2007, Hayward *et al.*, 2014) Recent years have seen more emphasis on research on tolerance as an explanation for high parasite loads in hosts with good body condition (Råberg, 2014). Thus if the pathogenicity of parasites is expected to be small and manageable, tolerance might be a worthwhile evolutionary strategy.

Host sex can have many different effects on parasite communities: males are usually more prone to parasite infections, have higher parasite loads and die younger due to infection (Zuk, 2009). Generally, the different hormone levels in males and females can lead to different immune responses (Roberts *et al.*, 2004). Nevertheless, testosterone or stress hormone levels are not the only sex differences. The sexes may differ in food consumption or movements, for example, more widely ranging males have higher parasite loads than more territorial females (Poulin, 1997; Schalk and Forbes, 1997). Life history trade-offs can additionally lead to different emphases in immune response during different times of the year (Schalk and Forbes, 1997).

Aggregation can also work at the interspecific level, i.e., some host individuals have more

parasite species than others (Poulin, 2007). The same host traits, which make the hosts more susceptible for parasites, can also make them more susceptible for acquiring more parasite species. Thus, intraspecific aggregation and interspecific aggregation can be positively correlated (Beldomenico *et al.*, 2008). There is nevertheless an additional dimension, interspecific competition, within host, and this could lead to less aggregated populations (Shaw *et al.*, 1998).

The message for the parasitologist is clear: if there is high aggregation, the sample needs to be large enough to get an accurate understanding of parasite load and abundance in the host population and the sample needs to be representative of the traits which we expect will affect aggregation (i.e., sex, body condition, age).

## 1.3.2. Parasite communities and coinfections within host

Co-infection is an increasingly important study subject as multiple infections is the norm in nature. The end results of co-infection to a host can be either elevated or reduced pathogenicity. Pathogenicity can be elevated, if parasites have a mutualistic relationship or if the first parasite facilitates the pathogenicity or the invasion of subsequent parasites. Easier concurrent colonization has been referred to as a "vicious circle", where parasite load leads to the poorer host condition decreasing resistance and allowing more parasites to colonize the host (Stephenson *et al.*, 2000; Beldomenico and Begon, 2010). If the co-infecting parasites are competing, the situation can also reduce pathogenicity (Bazzone *et al.*, 2008; Johnson and Hoverman, 2012).

For parasites, competition can lead to lower survival, slower growth, lower fecundity and shortened life span. The interspecific competition is normally asymmetric: some species might be minimally affected while it can have detrimental effects for others (Dobson, 1985; Knowles *et al.*, 2013). Host susceptibility can be modulated by existing parasites. Due to competition it might be more difficult to establish in an already-parasitized host, or vice versa and

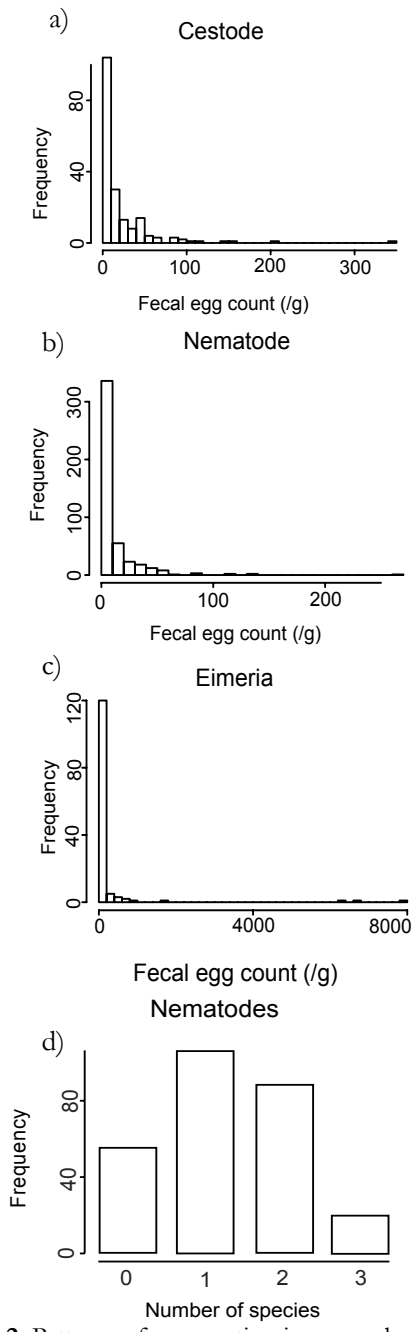


**Figure 2:** Patterns of aggregation in mouse lemur parasite communities as seen in my data. (a) Cestode, (b) nematode and (c) *Eimeria* egg shedding are aggregated (as they do not fit a Poisson distribution: variance-to-mean ratios 79, 44 and 471, respectively). Fecal egg counts though are not a reliable measure of infection intensity and actual number of adult parasites could be less aggregated (Stear *et al.* 1995; 2006) It should be noted, though, thatas shown in (d) the number of nematode species in mouse lemurs, there is no aggregation (sstudy III; VMR 0.60, difference from Poisson distribution: $\chi 2=211$, P267 = 0.99).

some species might be found more commonly together than otherwise (Cattadori *et al.*, 2007; Telfer *et al.*, 2010; Ezenwa and Jolles, 2011).

Are parasite communities random collections of species or are they more specific sets of regional parasites? The evidence is scarce, but studies uncovering community structures that diverge from random expectation are rare (Poulin, 2007; Pedersen and Fenton, 2007). The component community is often a subset of the regionally "available" parasites (Kennedy and Guégan, 1996; Mouquet *et al.*, 2003). The total number of species is limited either by the number of available niches - as new species can invade the community only by outcompeting existing species - or that the component community is not able to sample all the regionally available parasite species. At least in some cases the invasive species' parasites increase the diversity of the component community (Font, 1998) meaning that there are "free niches" for those parasites.

The infracommunities rarely contain the theoretical maximum numbers of parasite species, that is, the whole diversity of the component community. For example Kennedy and Guégan (1996) found eel infracommunities rarely contained more than three parasite species whereas the component communities were much more diverse. There are also contrasting results; Norton *et al* (2004) discovered a strong linear relationship between maximum infracommunity richness and component community richness. There is a limit on the total parasite biomass that each host individual can sustain (George-Nascimento *et al.*, 2004). Biomass should be taken into account when measuring parasite loads because rare species are often large. Thus when abundance is considered as biomass rather than the number of individuals, rare species are not necessarily as rare (Muñoz *et al.*, 2005). Nevertheless, parasitological work mostly uses the number of individuals as the proxy for parasite load.

## 1.4. Monitoring temporal variation in parasite communities

Monitoring temporal variation in parasite communities is inherently difficult. If there is cyclical variation, the host population needs to be followed preferably at least two cycles. Cyclical variation can have seasonal, annual or longer interval (Poulin, 2007). Furthermore, most often parasites are surveyed at the component community level, as this is easiest, even though the interactions always happen at the infracommunity level.

While mammal intestines are a relatively uniform and stable habitat, there are many factors driving temporal variation. Within hosts there is changing nutritional and immunological status, changing the quality of habitat for the parasites. Between parasites, there is density-dependent regulation of populations; parasite fecundity and survival could decrease due to competition or stronger host defenses when parasite loads increase. Between hosts, there is also density-independent regulation of parasite populations, including, for example, parasite-independent host population size variation: the carrying capacity for parasites is dependent on the host population size, behavior and seasonality.

While epidemiology is good at modelling host population–level processes and immunology has studied host-parasite interaction for a long time, how these infracommunity-level processes translate to component community-level outcomes is still poorly understood.

### 1.4.1. Temporal variation in infracommunities

As macroparasite communities are slow to respond to change, history plays an important role in the composition of parasite communities. Parasite load and diversity both increase with the age of the individual due to the accumulation of new parasites. This process continues until the host reaches a (dynamic) balance of infection and clearance (Schalk and Forbes, 1997).

Nevertheless, this balance varies between individuals and age groups. Young individuals have higher variability in fecal egg count (FEC) and the youngest and the oldest individuals have the highest FECs (Wood *et al.*, 2013). Sometimes so-called environmental age, cumulative environmental stress, is a better predictor for ageing effects than actual age (Hayward *et al.*, 2009).

The infracommunity is, from an evolutionary point of view, always ephemeral, as it is lost when the host dies. Macroparasites rarely have enough generations in relation to the life span of the host to allow for any local adaptation in infracommunities. The parasite age at maturity can vary widely, though it is normally dependent on the size of female with the smaller the parasite, the faster it reaches maturity (Poulin, 2007). The changes in parasite infracommunity, or the succession of communities, are not yet widely studied. We do not know how stable infracommunities are. At least some cestodes are able to live for years and many parasites can keep their host colonized through autoinfection. Thus there is at least a possibility for long-term stability.

Interactions between parasites are the shaping force in infracommunities and stronger interactions lead to more predictable groupings of species (Poulin, 2007). There is both intra- and interspecific competition between parasites. Parasites can also exert an influence after they have been cleared: for example immune memory can shape parasite communities (Lello *et al.*, 2008; Rynkiewicz *et al.*, 2015).

Poulin (2007) sketches four different modes of density-dependent regulation: exploitation competition, interference competition, host-mediated restriction and parasite–induced host mortality. Exploitation is the central property of parasites, as they need to compete for limited resources (Read, 1951; Bush and Lotz, 2000; Roberts, 2000). Interference is used by some parasites; for example cestode *Hymenolepis diminuta* secretes molecules that harm their competitors (Keymer, 1982). Host-mediated restriction can be due to the naivety of the immune defense upon first exposure and the mounted response during later exposures. Parasite-induced host mortality is a different mechanism leading to the destruction of the whole infracommunity and also affects host population dynamics. It should be noted, though, that there is no reason why every infracommunity should have density-dependent competition, as there appear to be cases where this does not happen (Marcogliese, 1997).

### 1.4.2. Temporal variation in component communities

Many component communities seem to have long-term stability (Haukisalmi *et al.*, 1988; Haukisalmi and Henttonen, 1990) or regular seasonal variation (Altizer *et al.*, 2006; Nalubamba *et al.*, 2012; Majekodunmi *et al.*, 2013), though long-term data is limited. Nevertheless, stochastic effects on the component communities might be so significant in comparison to ecological turnover that they completely mask the influence of the local habitat and the hosts (Poulin, 2007; Behnke *et al.*, 2008a). In any case, while infracommunity is dependent on host life span, component communities can theoretically be sustained as long as the host population exists. After a substantial anthropogenic change in ecosystem, including fragmentation or introduction of new potential hosts and their parasites, component communities are slow to change (Torchin *et al.*, 2003). Due to these reasons, the anthropogenic effects on component communities are poorly understood.

As metapopulations, parasite communities could be changing due to colonization-extinction dynamics (Ebert *et al.*, 2001; Lion and Gandon, 2014). Parasite communities are true metapopulations: infracommunities consist of individual populations and the component community is the union of all local metapopulations. Due to host death, extinctions are an ever-present feature of parasite dynamics, and thus colonization, i.e. infection, is central for parasite survival.

The core and satellite hypothesis of metapopulations predicts that the core parasite species remain stable whereas the other parasite spe-

cies are rare and only present locally (Nee *et al.*, 1991; Morand and Guégan, 2000; Guégan *et al.*, 2005). This leads to the idea that for evolutionary analysis, core species are the most important as they interact with each other and with their hosts constantly, while satellites contribute little to the structure of communities. For example, satellite species could be spillovers from other host species. Another possibility is nested sets of parasite species that are non-random sets dependent on the ability of communities to sustain parasite diversity: the core species occur everywhere whereas some of the parasite species occur only in diverse communities (Poulin, 2007). Considering community dynamics, this would be seen as the more stable occurrence of rare parasite species in host individuals that have high parasite diversity. Co-infection dynamics can also affect the structure of communities as multiple infections can be more or less common than expected depending on the parasites' and host's interaction (Alizon, 2013a; b; Viney and Graham, 2013).

It is notoriously difficult to identify species compositions that might have resulted from competition or cooperation in nature (Haukisalmi and Henttonen, 1998; Poulin, 2005; Behnke, 2008; Fenton *et al.*, 2010; Shrestha *et al.*, 2011; Vaumourin *et al.*, 2014). Together with the increasing data on parasite communities, there has also been a surge of new null models for parasite communities (Gotelli and Ulrich, 2012; Ulrich and Gotelli, 2013). The importance of the null models cannot be overemphasized as identification of non-random patterns and associations between parasite species is difficult (Gotelli and Colwell, 2001; Behnke *et al.*, 2005). Furthermore, departure from random expectation not only tells us about the parasites, but also about the hosts. Namely, it can reveal details about interspecific competition, heterogeneity in host susceptibility and the effect of intermediate hosts on community composition (Poulin, 2001). Nevertheless, considering parasite species in isolation could also lead to mistaken inferences (Telfer *et al.*, 2010).

## 1.5. Quantifying parasite communities

What do we want to measure, when we monitor parasite communities? I have mentioned previously, that the population of one parasite species might be too widely distributed – in mouse lemurs, insects and in soil – and we cannot sample all possible sites. For my thesis, I wanted to monitor parasite dynamics, so am primarily interested in the presence or absence of nematode species and their relative proportion in reference to the total parasite load of different species.

Presence and absence not only provides an estimate of parasite richness in the host individual, but in longitudinal setting I can detect if there is host colonization by new parasites or extinctions of parasite populations. Therefore it is the most important measure for longitudinal study. Potential measures of parasite loads, as mentioned previously, are not reliable indicators of parasite fitness or even for the host importance on parasite population reproduction (Eysker and Ploeger, 2000).

Parasite load is very difficult, and sometimes impossible, to quantify using non-invasive sampling as parasite load is defined as number of parasites within host individual (Jorge *et al.*, 2013). For gastrointestinal parasites, this is usually measured with invasive techniques such as the dissection of the gastrointestinal tract to count the number of adult parasites (Poulin and Morand, 2000).

Quantifying parasite eggs in fecal matter is the most common method of surveying gastrointestinal parasites non-invasively. Fecal egg count (FEC) is often used as a measure of parasite load – or at least as a proxy -, but there are obviously problems as FEC does not necessarily correlate perfectly with number of parasite individuals within host (Stear *et al.*, 1995, 2006; Gillespie, 2006). Positive or negative interaction between parasites can increase or decrease FEC, respectively (Bordes and Morand, 2011). Furthermore, the host condition and immune defense should have an effect on FEC (Dorchies *et al.*, 1997; Jolles *et al.*, 2008).

Proper and reliable identification of parasites is imperative to measure parasite dynamics. This is no easy task as there is a vast number of different parasite species and very few parasitologists who can identify them. Collecting samples is time-consuming as there are different protocols for different parasite taxa. The identification of parasite species requires adult individuals as many taxa are difficult to identify from their egg, cyst or larval forms or they need to be identified from sex-specific traits (Gasser, 2006). Adult individuals are not always available and assessments can only be performed on a more general level (Floyd *et al.*, 2002). Coproculture is laborious, unreliable and requires expertise to identify different species and developmental stages (Gasser, 2006).

At the moment, DNA sequencing is the preferred method for molecular biodiversity studies (Gasser *et al.*, 2008; Valentini *et al.*, 2009; Creer *et al.*, 2010; Bik *et al.*, 2012). Previously sequencing was used to detect or identify individual worms (e.g., Jenkins *et al.*, 2005; Asmundsson *et al.*, 2008; Kutz *et al.*, 2013; Budischak *et al.*, 2015), but it can also be used to screen multiple parasites (Tanaka *et al.*, 2014). Multiple sequences allow for identifying operational taxonomic units from parasite communities, makes sequencing of large sample sets possible and large databases make it possible to match template sequence to all published sequences.

## 1.6. Barcoding using next-generation sequencing

Traditional taxonomy is in many cases too slow for the needs of ecologists (Valentini *et al.*, 2009; Creer *et al.*, 2010; Abebe *et al.*, 2011; Bik *et al.*, 2012) and this has sparked debates between "taxonomists" and "end-users". Molecular biology has been presented as the solution for this impediment created by the perceived slowness of the taxonomical process (Brooks and Hoberg, 2001; Carvalho *et al.*, 2007; Emerson *et al.*, 2011; see Table 2). DNA can nowadays be routinely isolated from most known parasitic species (Caron, 2009) and sequence comparisons performed quite easily. Many new molecular methods are designed for quick and reliable diagnosis of humane or domestic animal pathogens, where accurate and swift identification is necessary (Ferri *et al.*, 2009). It can also be expected that with these molecular methods, the known biodiversity of the intestinal parasites will increase significantly as there will be sampling in previously unstudied environments and cryptic species separated (Caron, 2009).

Barcoding is a recent approach for species identification (Hebert *et al.*, 2003). The idea is simple: if we can sequence a gene, which has interspecific differences (which are true representations of phylogenetic relations) and minute or non-existent intraspecific differences, we should be able to assign any given individual to proper species from a DNA sample (Blaxter, 2004; Fig. 3). There are some additional requirements for a good marker gene: it should be sufficiently conserved to enable the design of universal primers, easily isolated and amplified, the sequence should be easy to align, there should be extensive genomic libraries available and the likelihood of intraspecific variation should be uniform across taxa. Additionally, it should have very low within-population and between-population variation and measureable between-taxon variation.

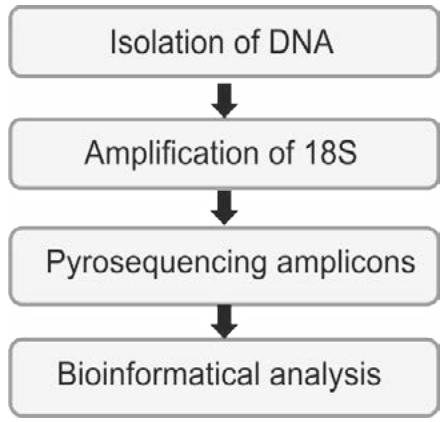


**Figure 3:** Workflow for metabarcoding. First DNA needs to be isolated from the samples, then the desired gene region (in my case 18S) needs to be amplified with PCR and then these amplicons will be sequenced. The resulting sequences need to be analysed through bioinformatical pipelines that need to deal with sequencing errors and assign sequences to reliable taxonomic identifications.

Table 2: A partial list of proposed benefits of the barcoding approach to the parasite identification.

| Benefit | Examples |
|---|---|
| All life stages can be identified | Leung *et al.*, 2009, Locke *et al.*, 2011 |
| Cryptic species recognized | Ferri *et al.*, 2009; Ogedengbe *et al.*, 2011 |
| Molecular methods make high-throughput processing of samples possible | Not yet done, but see Ferri *et al.*, 2009 |
| Identification of wide range of parasites | Not yet done, but see Deagle *et al.*, 2009 |
| Use of bulk environmental samples. | Not yet done, but see Deagle *et al.*, 2009 |
| Homology of genes is easier to predict than homology of morphological characters. | Under debate, see Silva *et al.*, 2010 |
| DNA sequences are digital and easy to communicate from laboratory to laboratory | Routine procedure. |

The current gene of choice for metazoans is cytochrome oxidase I (COI or coxI) (Hebert *et al.*, 2003). It has been successful with many taxa, though it is now clear that coxI does not work for every phylum. Standardization is progressing slowly in these difficult phyla (Frézal and Leblois, 2008) and not all species inside otherwise well-functioning phyla can be amplified (Santos *et al.*, 2011). COI does not work well with nematodes, and 18S is a more commonly used marker gene (Porazinska *et al.*, 2009; Tanaka *et al.*, 2014)

Nevertheless, species identification using barcoding requires extensive and reliable databases of targeted species. Even though sequencing is used to create vast amounts of sequence data, parasite species identification needs to be based on morphological data and thus voucher samples are imperative so identifications can be verified (Brooks *et al.*, 2014). This also allows for the validation of next-generation sequencing techniques, as they can be compared to results using traditional Sanger sequencing (Hudson, 2008). While species delimitation and species identification are two different problems, integrated approaches can solve both (Kutz *et al.*, 2007). Furthermore, an integrated approach adding spatio-temporal data on parasites, hosts and ecosystems to molecular and morphological data provides a robust framework to explore parasites species and their impact (Hoberg, *et al.* 2015).

Next-generation sequencing allows surveying of environmental samples (i.e., samples which are total genomic samples from many different species and individuals) with speed, precision and lower cost per base compared to traditional Sanger sequencing. Barcoding environmental samples using high-throughput sequencing platforms is called metabarcoding (Bik *et al.*, 2012). Pyrosequencing, a "sequencing by synthesis" -based sequencing approach, is based on the detection of pyrophosphate released due to the addition of nucleotides to the DNA strand and can detect multiple sequences from a single complex sample (Casiraghi *et al.*, 2010). With these advances researchers are able to work much faster as sequenced individuals do not need to be separated from the original sample.

Using cellular debris as the target material, fecal metabarcoding started with microbial and dietary samples. There are also evident problems, for example, fecal barcoding with all metazoans has proved to be difficult and quantitatively unreliable in diet analysis barcoding (Symondson, 2002; King *et al.*, 2008; Deagle *et al.*, 2009). While free-living nematodes have been studied with this method (Porazinska *et al.* 2009, 2010a; b, 2012), parasitic nematodes are rarely studied (Tanaka *et al.*, 2014).

Intestinal parasites normally have abundant amounts of DNA among the feces (in the form of eggs, cysts and body parts) but, in cases where genetic material is rare fecal samples need to be treated carefully, because rare material can be lost in the first steps of PCR (Jarman *et al.*, 2004). It is essential to develop more effective

methods to extract parasite DNA from feces, as high-throughput method rely on the usability of fecal DNA (Prichard and Tait, 2001). Also, conventional barcoding uses long sequences (~600 bp) but environmental samples may contain degraded DNA where 150bp would be nearer the average length (Valentini *et al.*, 2009). High degradation rates are problematic as higher quality DNA amplifies better and host DNA tends to be of higher quality than degraded parasite DNA. DNA-based methods – even using the environmental approach – cannot be quantitative (Gasser, 2006). Quantitative analysis from feces is exceedingly difficult as the amount of eggs in feces varies daily and can only be used as an indicator in prolific egg layers (Prichard and Tait, 2001).

# 2. Aims of thesis

This thesis makes advances on two fronts: methodological development for parasite identification by metabarcoding and in parasite community ecology investigating the temporal dynamics of parasites in wild host populations. More specifically, this thesis addresses the following questions:

- How can we robustly identify intestinal nematode species from the fecal samples of mammals (Studies I, II)

- How does the temporal dynamics of macroparasites differ at the infra- and component community levels? (Study III)

- Do life history traits or habitat differences explain infracommunity variation and dynamics? (Studies III, IV)

- Do sympatric host species share same parasite species? (Study I)

Based on a review of research literature I formulated hypotheses for each of these research questions (Table 3). Some of these expectations differ depending on competing theories, e.g., is the component parasite community governed by core-satellite dynamics or are they nested sets while some are dependent on species-specific traits, e.g. do mouse lemurs invest more in tolerance or in resistance.

The first article outlines a new high-throughput method for parasite community surveys and validates the approach by using rodent samples collected from Madagascar by myself and earlier samples collected by Lehtonen (*unpubl.*) that have been morphologically identified to the species- or genus-level. I also compare the composition of parasite communities in sympatric rodents and discusses the overall challenges of the methods employed. The second article describes the Séance pipeline, which combines both existing and new bioinformatics tools for the analysis of amplicon data.

The third and fourth articles handles the mouse lemurs and their parasites at the individual and population-level. The third article looks at nematodes in component and infracommunities, that is, at the population-level and the individual-level, over a period of three years. The fourth article examines at the correlation between mouse lemur body condition and different groups of parasites.

**Table 3:** Derived hypothesis and expected results for my thesis.

| Question | Factors | Known effects | Expectation |
|---|---|---|---|
| Differences in temporal dynamics of infra- and component communities | Stability | Component community more stable than infracommunity | Component community more stable than infracommunity |
| | | In long-living species, infracommunity has long-term stability | Infracommunity stable |
| | Seasonal variation | Increase in rainfall leads to more insects and thus more parasites | Component community gets more diverse after the beginning of the field season |
| | | Hibernation leads to parasite extinction | |
| | Common vs. rare parasites | Core-satellite –hypothesis | Common species stable |
| | | | Rare species randomly occurring |
| | | Nested sets -hypothesis | Common species stable |
| | | | Rare species in hosts with more diverse parasite community |
| Life history traits and habitat differences in infracommunity composition and dynamics | Sex | Higher testosterone level leads to weaker immune response | Males and females have similar parasite loads |
| | | More movement leads to higher parasite diversity | Males have higher parasite diversity |
| | Age | Weaker immune response leads to higher parasite loads | Young individuals have higher parasite loads |
| | | History affects infracommunities | Older individuals have higher parasite diversity |
| | Site | Degraded area is poorer habitat | Leads to lower body condition |
| | Body condition | Higher body condition leads to higher resistance OR | With better body condition, lower parasite loads |
| | | Higher body condition leads to higher tolerance | With better body condition, higher parasite loads |
| | Social system | Social connections lead to higher parasite transmission | Males have higher parasite loads and diversity |
| | Hibernation | Hibernation leads to parasite community extinction | Early in season less diverse parasite community |
| | | Males hibernate less than females | Males have higher parasite diversity |
| Sharing of same species by sympatric species | Exotic species | Host switches between endemic species | Exotic species have some but not all endemic parasite species |
| | | | Endemic species acquire new species |
| | High host diversity | High diversity of niches can sustain more parasite species | Comparably higher parasite numbers than in other tropical areas |
| | Size | Smaller species have more parasite species | Dogs and bigger lemurs have more parasite species than rodents |
| | Phylogeny | Closely related host species share same parasite species | Lemurs share same species |
| | | | Rodents share same species |
| | Habitat | Similar habitat leads to sharing same parasite community | Terrestrial species share parasite species |

# 3. Material and methods

## 3.1. Study species

### 3.1.1. Hosts

#### *3.1.1.1. Mouse lemurs*

Most of my samples are from rufous mouse lemurs (*Microcebus rufus*, Fig. 4a), one of the smallest species of primates in the world. Rufous mouse lemurs' weigh between 30 and 100 grams and are approximately 10 centimeters long excluding the tail. (Atsalis, 1999) Mouse lemurs belong to the suborder Strepsirrhini, like all primates from Madagascar (Mittermeier *et al.*, 2008).

Mouse lemurs are omnivorous, though they prefer insects to plant material. During the dry season, when insects are less abundant, mouse lemurs eat a variety of plant material from buds and leaves to nectar, gum and fruit (Atsalis, 2008). Mouse lemurs are nocturnal and forage alone in the small branches of the canopy avoiding ground level (Atsalis, 2008; Joly and Zimmermann, 2011). Mouse lemurs apparently share their sleeping nests, but the patterns of their social interaction are still poorly understood (Zohdy *et al.*, 2012). Males seem have a greater range than females (Joly and Zimmermann, 2011). Mouse lemurs are also able to torpor in case of adverse conditions or even hibernate for longer periods. Females tend to hibernate all through the dry season whereas males hibernate for a shorter period (Schmid, 1998; Schülke and Ostner, 2006; Atsalis, 2008; Kobbe and Dausmann, 2009).

The mouse lemur populations in Ranomafana National Park breeds once a year in October just before the wet season (Blanco, 2008). The females are receptive – as their vulvas are open - for only one or two nights during the breeding season (Blanco, 2011). They mate multiple times with multiple males during that period. The males' testes are very small for most of the year but they start to grow before the mating season, receding afterwards. After a gestation period of 56-58 days, mouse lemurs give birth to 1-3 infants and they wean their offspring after 40 days (Blanco, 2008, 2011).

I opportunistically sampled bigger-sized lemurs including brown lemurs (*Eulemur rubriventer* and *E. rufifrons*) and bamboo lemurs (*Hapalemur aureus* and *Prolemur simus*). All these species are dominantly diurnal and live in small groups, mostly eating plant material.

#### *3.1.1.2. Rodents*

I caught rodents both while trapping mouse lemurs and using specially designated traps on the ground. Of the endemic rodents I caught red forest rats (*Nesomys audeberti* (Fig. 4) and *N. rufus*) and tufted-tailed rats (*Eliurus webbi, E. tanala* and *E. minor*). Red forest rats are medium-sized rodents weighing between 140 and 300 grams and they forage diurnally (Garbutt, 2007). They are poor climbers and tend to restrict themselves to the forest floor (Ryan *et al.*, 1993). In comparison, tufted-tailed rats are smaller, 30-100 grams, and are mostly nocturnal and arboreal (Carleton, 2003). Tufted-tailed rats prefer closed and dense forests.

My trapping also included one invasive rodent species, the black rat (*Rattus rattus*). The black rats are generally smaller than continental conspecifics, between 80 and 150 grams. The black rats in Madagascar tend to be human commensals and are restricted to urban and agricultural areas (Garbutt, 2007). The black rats as a general rule do not go more than 500 meters into the forest (Lehtonen *et al.*, 2001; Lehtonen, 2013). Their main nutrition in Madagascar is human food or leftovers from plantations. The black rats probably followed humans to Madagascar 1000-2000 years ago (Hingston *et al.*, 2005; Tollenaere *et al.*, 2010).

**Figure 4**: Sampled host animals, from left to right, mouse lemurs, red forest rats and frogs.

### *3.1.1.3. Other species*

I also collected samples opportunistically from semiferal domestic dog (*Canis lupus familiaris*). The dogs tend to keep to the human settlements and are rarely encountered in the forest. The dogs are omnivorous and tend to be the top predators in their ecosystem. The dogs came with humans to Madagascar but their arrival time has not been pinpointed.

Furthermore, some of the ground-placed traps also trapped snails weighing 100-200 grams. The snails looked morphologically similar, but I was not able to identify the species.

Kendall Harris (Sweet Briar College, USA) did her Study Abroad independent project on the parasites of frogs. She collected fecal samples from several different frog species, the two most common being *Ptychadena mascariensis* and *Mantidactylus lugubris* (Fig. 4). Both are small-sized, 5 to 30 grams, diurnal frogs living near streams on the ground or in low-hanging branches of trees. Frogs are restricted to invertebrate prey, mostly insects. *Ptychadena* belongs to a Sub-Saharan African group of frogs whereas *Mantidactylus* is restricted to Madagascar and Mayotte. The closest relatives of *Mantidactylus* outside Madasgascar can be found from the Indian subcontinent. Both of the ancestral species have probably been in Madagascar before the break-up from Gondwana 110 million years ago (Vences *et al.*, 2003, 2004; Vences, 2004; Samonds *et al.*, 2012).

## 3.1.2. Parasites

All parasites in the study are either intestinal parasites or ectoparasites (Table 4). Both groups are strongly dependent on their host individuals. In reference to the earlier definition of the parasite (section 1.1.1), all of these species are definitely symbiotic to their hosts. Lice suck the blood of their hosts to grow and lay eggs and cannot survive without contact with the host. Thus they can only change host individual if both hosts are in direct contact (Zohdy *et al.*, 2012). The female ticks suck blood from the lemurs and then drop to the vegetation to molt or lay eggs and then attach to their next host.

The gastrointestinal parasites can be divided into three distinct groups: eimeriids, cestodes and nematodes. Eimeriids (Fig. 5) are unicellular apicomplexan parasites, which cause coccidiosis in their hosts (Ogedengbe *et al.*, 2011). Eimeriids are important parasites of vertebrates, including domestic animals, and they regularly cause massive epidemics in dense populations. High numbers of eimeriids can cause gastrointestinal pain, intestinal bleeding and diarrhea (Mykytowycz, 1962). Eimeriids have direct lifecycles and are spread via the fecal-oral route.

Cestodes are a group of parasitic flatworms (phylum Platyhelminthes, Fig. 5), including many human parasites known as tapeworms. Cestodes attach to the intestinal lining with their head part (scolex) and collect nutrients from the food

**Table 4:** The complete list of parasites encountered from the samples collected during this study.

| Parasite group | | Parasite species | Host | Detection | Studies |
|---|---|---|---|---|---|
| Ectoparasites | Lice | *Lemurpediculus verruculosus* | Mouse lemurs | Visually from ears | IV |
| | Tick | *Haemaphysalis lemuris* | " | " | " |
| Intestinal parasites | Nematode | Putative species 1 – "*Strongyloides*" | Mouse lemurs, gastropods, frogs, black rats | Baermann's | I, III, IV |
| | | PS2 – "*Caenorhabditis*" | " | " | " |
| | | PS3 – "Strongylida" | Dogs, all lemurs, black rats | " | III, IV |
| | | PS4 – "Chromadorea" | Mouse lemurs | " | " |
| | | PS5 – "*Enterobius*" | " | " | " |
| | | PS6 – "*Panagrellus*" | Mouse lemurs, dogs, black rats | " | I, III, IV |
| | | PS7 – "Rhabditoides" | Frogs | " | Harris *et al.*, in prep |
| | | PS8 – "*Raillietnema*" | " | " | " |
| | | PS14 – "*Phasmarhabditis*" | Gastropods | " | Aivelo *et al.*, in prep |
| | Cestode | *Hymenolepis nana* | Mouse lemurs | Fecal flotation | IV |
| | | *Hymenolepis diminuta* | " | " | " |
| | Eimeriids | | " | " | Aivelo *et al.* in prep |

material digested by the host. Their bodies are composed of segments (proglottids) that contain sexual organs. Fully-grown cestodes can be tens of centimeters in length and lay thousands eggs per day (Haukisalmi *et al.*, 1998). The host specificity of cestodes is debated, but at least several species are able to infect multiple hosts (Poulin and Keeney, 2008). The cestodes can be spread via the fecal-oral route or they can have intermediate hosts, for example, the insects mouse lemurs prey on. Mouse lemur can be the definitive host of cestode or an intermediate host, as they are prey to the larger predators.

Roundworms, or nematodes, are a vast phylum of mainly small-sized worms that can be ectoparasites, intestinal parasites or endoparasites (Fig. 5). I have collected all available data on previously sampled nematodes on mouse lemurs and black rats in Table 5. Nematodes are generally attached to the intestinal lining and they are either feasting on the intestinal mass of bacteria and other nutrients or sucking nutrients from the intestinal wall. Nematodes can spread through different routes: those with direct life cycles via the fecal-oral route or by free-living stages in the soil. There are also species with complex life cycles containing one or two intermediate hosts. Many species are able to sustain their intestinal populations via autoinfection. For example, *Strongyloides* larvae can penetrate the intestinal wall, move through the circulatory system, invade the lung tissue and re-enter the gastrointestinal tract when the mucus secreted from the lungs is swallowed (Sandground, 1926; Nishigori, 1928).

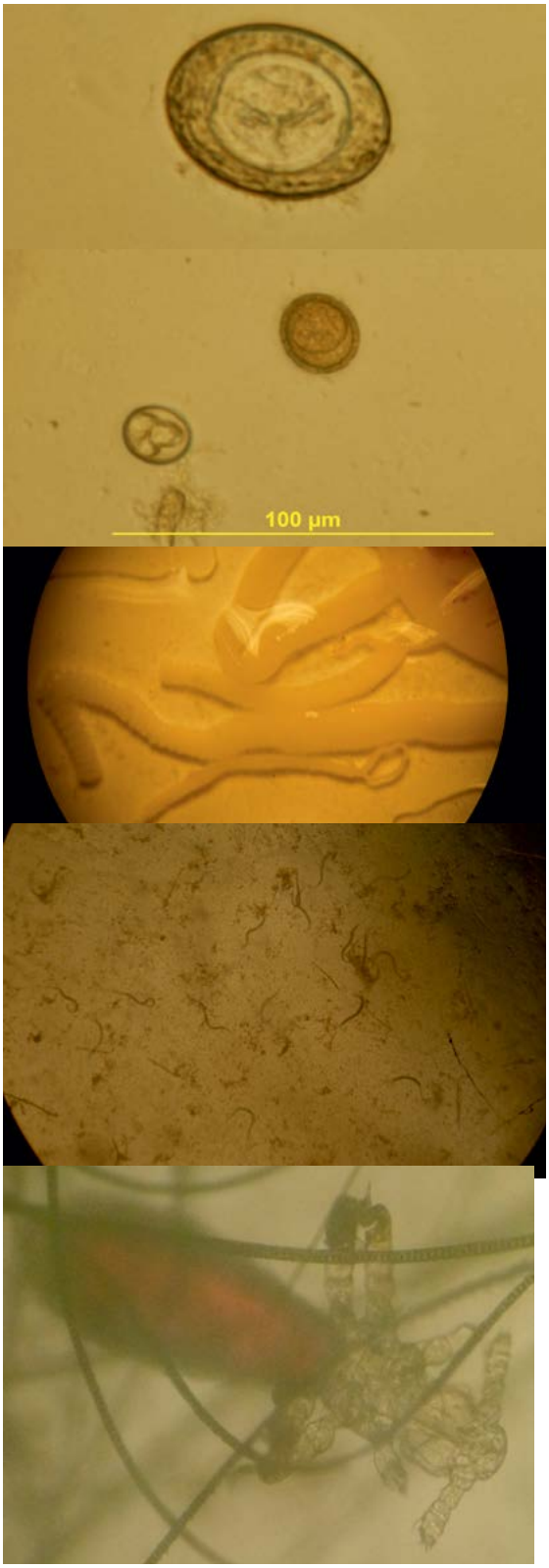


**Figure 5:** Sampled parasites include, from top to bottom: cestode eggs, Eimerias, adult cestodes, nematodes and lice.

I have not been able to ascertain whether the presence of these parasite species lead to actual expense for their hosts. While I assume they are parasites, it should be noted that this is a general assumption in parasitological research. There are no known mutualistic intestinal nematodes, cestodes or eimeriids. The ectoparasites can be safely assumed to impose an expense as they suck blood from their hosts, but it is not clear how high this expense is.

## 3.2. Study area: Ranomafana National Park

Ranomafana National Park is a protected area in southeastern Madagascar (21°16' S latitude and 47° 20' E longitude) that consists of 435 km2 of continuous montane rainforest (Fig. 6). The elevation of the park varies between 500 and 1500 meters above sea level. The park was established in 1991 and the area was partially selectively logged prior to that (Wright and Andriamihaja, 2002). The park is surrounded by three kilometer wide buffer zone. The National Park is also home to a research station Centre Valbio, where my laboratory space was situated (Wright *et al.*, 2012). The area has dry and cold weather during the austral winter and warm and rainy weather in austral summer (Table 6, Fig. 6).

I had two different transects: Talatakely and Campsite (Fig. 6). The Talatakely transect was just inside the National Park. The area is protected but it was selectively logged during the 1980s and is considered secondary forest. There are no high trees (max. height 10 m) but there is rather extensive shrub-like undergrowth. The Talatakely transect begins at the entrance of the park and is in heavy use by tourists. Local mammals and birds suffer from the stress caused by tourists (Herrera *et al.*, 2011; Wright *et al.*, 2014). Tourists are not allowed to visit the park after 4 pm, so the effects to mouse lemurs are mostly indirect. The second transect was on the peripheral zone of the park in the campsite of the research station. The area was clear-cut several years prior to my study and consists of short trees and shrubs with non-continuous canopy cover. The area is frequented by local farmers

**Table 5**: Nematodes surveyed in Madagascar from either mouse lemurs (Microcebus murinus, M. ravelobensis) or black rats (Rattus rattus)

| Host | Parasite order | Species | Location | Reference |
|---|---|---|---|---|
| Microcebus murinus | Rhabditidae | *Strongyloides* sp. | Mandena | Raharivolona, 2006 |
| | Strongylida | *Pararhabdonema longistriata* (?) | " | " |
| | | Strongylida sp. | " | " |
| | | | Ankarafantsika | Radespiel *et al.*, 2015 |
| | Oxyurida | *Lemuricola microcebi* | captive | Hugot *et al.*, 1995 |
| | | *Lemuricola* sp. | Mandena | Raharivolona and Ganzhorn, 2010 |
| | | | Kirindy | Schwensow, *et al.* 2010 |
| | | | Ankarafantsika | Radespiel *et al.*, 2015 |
| | | *Enterobius* sp. | Mandena | Raharivolona, 2006 |
| | | Oxyurida sp. | " | Raharivolona and Ganzhorn, 2010 |
| | Ascaridida | *Subulura baeri* | Ampijoroa, Mananara | Chabaud *et al.*, 1965 |
| | | *Subulura* sp. | Mandena | Raharivolona, 2006 |
| | | | Kirindy | Schwensow, *et al.* 2010 |
| | | | Ankarafantsika | Radespiel *et al.*, 2015 |
| | | *Ascaris* sp. | Mandena | Raharivolona, 2006 |
| | | | Kirindy | Schwensow, *et al.* 2010 |
| | | | Ankarafantsika | Radespiel *et al.*, 2015 |
| | | Ascaridida sp. | Mandena | Raharivolona, 2006 |
| | Spirurida | *Spirura diplocyphos* | Ampijoroa | Chabaud *et al.*, 1965 |
| | | *Rictularia lemuris* | " | " |
| | | *Dipetalonema petteri* | " | " |
| | Enoplida | *Trichuris* sp. | Mandena | Schad *et al.*, 2005 |
| | | *Capillaria* sp. | " | Raharivolona, 2006 |
| | | *Trichosomoides* sp. | " | Schad *et al.*, 2005 |
| M. ravelobensis | Strongylida | Strongylida sp. | Ankarafantsika | Radespiel *et al.*, 2015 |
| | Ascaridida | *Subulura* sp. | " | " |
| | | *Ascaris* sp. | " | " |
| | | *Lemuricola* sp. | " | " |

**Table 5** continued

| Host | Parasite order | Species | Location | Reference |
|---|---|---|---|---|
| Rattus rattus | Rhabditidae | *Strongyloides ratti* | Ranomafana | Lehtonen, unpubl. |
| | | *Strongyloides* sp. | Mandena | Raharivolona *et al.*,, 2007 |
| | Strongylida | *Nippostrongylus brasiliensis* | Ranomafana | Lehtonen, unpubl. |
| | | *Angiostrongylus cantonensis* | Antananarivo | Breuil and Coulanges, 1982 |
| | | *Trichostrongyloidae* sp. | Mandena | Raharivolona *et al.*,, 2007 |
| | | Strongylida sp. | ” | ” |
| | Spirurida | *Mastophorus muris* | Ranomafana | Lehtonen, unpubl. |
| | | *Spirurida sp.* | Mandena | Raharivolona *et al.*,, 2007 |
| | Ascaridida | *Heterakis spumosa* | Ranomafana | Lehtonen,unpubl. |
| | | *Ascaris* sp. | Mandena | Raharivolona *et al.*,, 2007 |
| | | Ascaridida sp. | ” | ” |
| | Oxyurida | *Syphacia muris* | Ranomafana | Lehtonen, unpubl. |
| | | *Enterobius* sp. | Mandena | Raharivolona *et al.*,, 2007 |
| | | Oxyurida sp, | ” | ” |
| | Enoplida | *Trichuris* sp. | ” | ” |
| | | *Capillaria* sp. | ” | ” |

**Table 6:** Rainfall patterns during the study. Years 2010 and 2012 had high precipitation during the dry season (April to July in 2010: 1074 mm, 2011: 457 mm, 2012: 1103 mm), while 2011 had high precipitation during the interval from dry to wet season (August to December, 2010: 571 mm, 2011: 1246 mm, 2012: 1016 mm).

| Month | Rainfall in mm | | |
|---|---|---|---|
| | 2010 | 2011 | 2012 |
| January | 675 | 531 | 696 |
| February | 262 | 1167 | 890 |
| March | 820 | 441 | 672 |
| April | 200 | 242 | 558 |
| May | 253 | 72 | 223 |
| June | 188 | 59 | 243 |
| July | 433 | 84 | 79 |
| August | 188 | 216 | 19 |
| September | 40 | 146 | 243 |
| October | 79 | 125 | 127 |
| November | 96 | 319 | 383 |
| December | 168 | 440 | 244 |

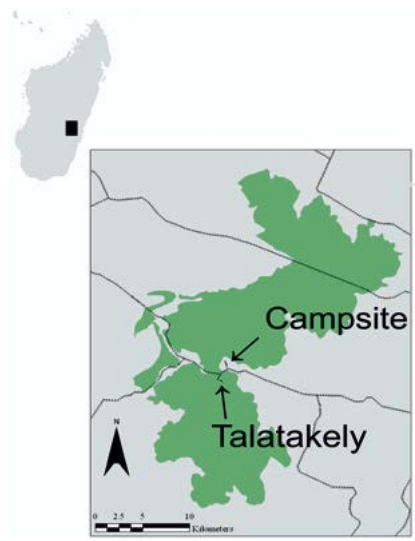


**Figure 6:** My study sites are situated either inside (Talatakely) or on the peripheral zone (Campsite) of Ranomafana National Park. Figure modified from Brooks *et al.* (2009).

who walk through the area to return home and by students staying at the campsite. I observed a high number of black rats and semiferal dogs. My two transects were separated by the river, which is assumed to be a dispersal barrier and thus my study populations are two distinct populations.

I acquired additional samples from fragmented forests in the peripheral zone of the park for black rats and greater bamboo lemur. Frogs were collected near the village of Ambatolahidimy in the peripheral zone of the park, about one kilometer from the campsite.

## 3.3. Sample collection and parasite survey pipeline

I divided collected faeces into several portions. One part was stored in RNAlater for microbiome study. One part was designated for the Eimeria and cestodes flotation: the feces were suspended in potassium dichromate and after 10 days, the sporulated eimeriids and cestode eggs were counted in 40% magnesium sulfate flotation. I got several adult cestodes expelled by mouse lemurs and these were used to validate the identifications performed by egg morphology.

The complete nematode survey pipeline is detailed in study I, Séance, a novel bioinformatics pipeline, for the analysis of 454 data, is detailed in study II.

I collected samples yearly from 2010 to 2012 after the dry season (August-September) and ended before the cyclone season hit the island (November-December) (Table 6). My study periods coincide with the mating season of mouse lemurs when they are the most active (Atsalis, 2008). I trapped mouse lemurs with Sherman traps, microchipped the individuals for identification on recapture, measured morphometrics and collected parasite samples (Figure 7, see details in study III)

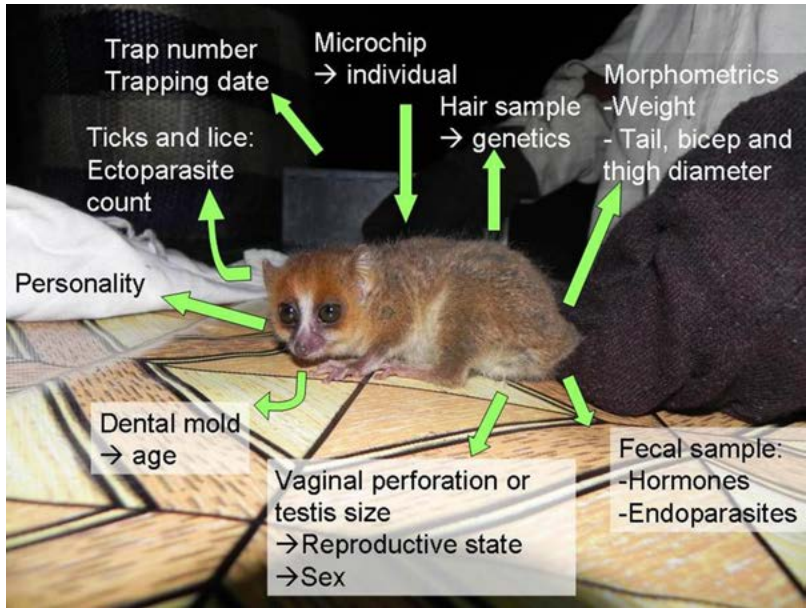


**Figure 7**: The different measurements and samples taken from each mouse lemurs. The dental molds, hair samples and some of the biometrics wastaken once a year, whereas parasite sampling, genital status, weight and personality were recorded on every catch.

## 3.5. Ethical considerations

I performed my research under strict regulations from Madagascar and University of Helsinki. The fieldwork was approved by the Viikki Ethical Committee at University of Helsinki and by the trilateral commission in Madagascar (permissions: 115/10/MEF/SG/DGF/DCB.SAP/SCBSE , 203/11/MEF/SG/DGF/DCB.SAP/SCBSE and 203/12/MEF/SG/DGF/DCB.SAP/SCBSE). The invasive procedures were limited to the microchipping of the mouse lemurs, as longitudinal studies require that the identification of individuals be performed with minimal interference on the study subjects.

To provide validation for my methods, I dissected invasive black rats and collected their intestinal nematodes. This was an optimal procedure, as the invasive species were in any case terminated as per the request of the Madagascar National Parks. The rats were dissected in the infectious disease labs in Centre Valbio using appropriate protective clothing. The cadavers were destroyed on-site.

## 3.6. Access to data and supplementary information

The nematode samples, residues of feces from Baermann's method stored in formalin, the fecal samples stored in ethanol, hair samples and teeth molds have been stored at the Institute of Biotechnology, University of Helsinki.

All sequence data has been released into the Sequence Read Archive under project number SRP042187. The corresponding accession numbers can be found in the metadata files for study I (doi: 10.6084/m9.figshare.1304408) and III (doi: 10.6084/m9.figshare.1108080), which include all data used in these studies. The data and accession numbers for Kendall *et al.* (2015) can also be found in Figshare (doi: 10.6084/m9.figshare.1309923). The phylogenetic data for study I can be found in TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S17092

The tutorial for Séance is published on the website of Ari Löytynoja's lab: http://wasabiapp.org/software/seance/

# 4. Main results and discussion

## 4.1.Method for the high-throughput sequencing and nematode survey (studies I-III)

I designed and implemented a new pipeline for the survey on intestinal nematodes that included all necessary steps from fieldwork to the analysis of sequences. Study I described and validated the complete pipeline, whereas study II presented, Séance, bioinformatics pipeline that combines several previously described programs in addition to original parts.

How can the suitability of this pipeline be assessed? The natural baseline is to compare my putative species to the nematode communities uncovered by previous studies (Table 5). While the number of recovered putative species was comparable to previous studies (see 4.2.), the communities differed. In general, I recovered more strongylid and rhabditid putative species, but I did not find any ascarid sequences. This probably reflects not only the parasite community but also the lack of discrimination in the 18S gene. Thus the identifications I have done should be viewed not as taxonomic identifications of parasite species but rather higher taxonomical level groupings.

There is a lack of parasitological studies in Malagasy small mammals and their parasite communities are poorly understood. Thus they are not an optimal system with which to develop new metabarcoding methodologies. A full validation of the method would a need more complete, integrated approach with longer-term trapping of hosts, collecting parasites both in egg and larval form from the feces and adult specimens from the host gastrointestinal tract. It should also be remembered that Baermann's method recovers only live larvae. In my studies, I investigated a subset of leftover feces samples to assess if there were eggs or larvae that were not extracted by the pipeline. I did not find a significant number of these. In other parasite systems, the situation can be different and my method might be unsuitable.

I used rodent samples to validate the method. Amplification proved to be difficult: even though the primers were universal for nematodes, the general success in sequencing from rodent fecal samples was quite low at 51%, while mouse lemur samples had a success rate of 44%. The identifications from adult samples and their eggs matched the same species, except for Mastophorus sp., which was not detected in the feces, only in adult samples. Across all studies, I delimited 9 putative species of intestinal nematodes from different host animals (Table 4) and an additional five putative species from control samples.

Nematodes are difficult to amplify and sequence. This is due to difficulties isolating DNA because of cuticle, low numbers of cells and small quantities of genetic material. In addition, fecal samples include a high number of inhibiting substances that makes amplification prone to failure. Another key problem is the lack of reliable universal primers for nematodes. However, this did not seem to be a critical problem as when I had ample genetic material (e.g., from larger-sized *Mastophorus, Toxocara, Parascaris*), sequencing success rates were higher. Nevertheless, using several different primer pairs might make the sequencing more reliable.

A new analysis method based on the phylogenetic placement of the amplicons was created to analyze the data. The reference phylogeny was built a priori using the complete 18S gene sequence from all nematodes in the SILVA database (Quast *et al.*, 2013). Thus the inferred phylogeny for the amplicons can lead to substantially better parasite identification than with crude BLAST-based methods. While a large number of unique sequences were discarded, those retained accounted for over 97% of amplicons that passed quality control. I expect that the excluded sequences contain errors from either amplification or sequencing.

The clusters delimited by sequence differenc-

es are not necessarily representative of species. This leads to a problem: what is the biological significance of this concept? This question is related to the role of pragmatism, as OTUs have been described as a "non-idealist" choice for accessing many taxon groups otherwise not studied (Blaxter, 2004). Only further studies will tell us the relationship between OTUs and species (Abebe *et al.*, 2011).

There are definite problems with the concept of barcoding. First of all, most species are not yet described, much less reliably sequenced and found in curated databases (Wilson *et al.*, 2011). Using placement on reference phylogeny can resolve this partially: differentiation between species can be done even though they cannot be identified precisely. The second problem is much more difficult: there are no truly universal primers for nematodes. While my primers seemed to successfully amplify nematode species, but for many of them, the resolution of the 18S gene region is too low to differentiate them even at the genus level. This problem can be overcome using longer amplicons or several primers, so called primer cocktails, to get at least some diagnostic gene regions sequenced (Prosser *et al.*, 2013). This makes the methods difficult to design and validate. The small differences in the target sequence might also cause additional problems. Depending on the sequencer, error rates on sequences can be quite high and it should also be taken into account as errors can lead to inflated estimates of diversity (Meyer and Paulay, 2005; Quince *et al.*, 2011). Sequencing multiple gene targets could be necessary to successfully barcode all of the nematodes present within one study.

Metabarcoding is enticing because it is relatively straightforward irrespective of taxa studied (Goldstein and DeSalle, 2011). However, as with any major scientific endeavor, it has not been without challenges and critics. Most debate is based on a "traditional taxonomy" versus barcoding argument (Ebach and Holdrege, 2005; Mitchell, 2011) and directed towards "DNA taxonomy" - a method of building a new taxonomic framework. Delimitation of new species based on DNA only has proved to be a highly contentious issue, though DNA identification of previously described species is more widely accepted (Lee, 2004; DeSalle *et al.*, 2005). Barcoding is a useful tool for ecological and evolutionary studies but it cannot substitute taxonomical, biogeographical or phylogenetic work on intestinal parasites (Perkins *et al.*, 2011). Nevertheless, the method shows a tangible approach to investigate parasite communities that are poorly understood and predominantly contain undescribed species.

Many of the problems I encountered are not related to barcoding, but rather to non-invasive surveys of parasites in general. Fecal analysis is an indirect method of identifying parasites, i.e., it can only detect those parasites that are laying eggs in the intestine. Thus, fecal egg counts cannot be used as measures of infection intensity. Fecal analysis is also known to be a less sensitive method for identification of helminths (Jorge *et al.*, 2013). Nevertheless, there are a lot of situations when invasive sampling is not feasible, for example, with endangered species, in longitudinal studies and when killing an animal would be considered unethical in comparison with the data acquired. Although not comparable to intestinal studies, the method shows a promise in making non-invasive sampling more sensitive and easier to perform.

## 4.2. Parasite community in Ranomafana National Park (studies I,III)

The number of putative nematode species in my study was comparable to previous studies performed in Madagascar on mouse lemurs. While I found 6 species in *Microcebus rufus*, Schwensow *et al.* (2010) found 7 species from M. murinus in Kirindy and Radespiel *et al.* (2015) 4 species from both *M. murinus* and *M. ravelobensis* in Ankarafantsika. Studies on *M. murinus* from Mandena provide highly discordant numbers: Schad *et al.* (2005) found 17 species or morphotypes, Raharivolona (2006) found 13 and the most recent study with the highest sample number only 6 (Raharivololona and Ganzhorn, 2009, 2010;

Radespiel *et al.*, 2015) All these studies were performed with fecal sampling due to the lack of opportunistic necropsies of mouse lemurs. There are only a small number of invasive parasitological studies investigating lemurs, but my results seem concordant with those studies (Irwin and Raharison, 2009; Clough, 2010; Clough *et al.*, 2010). Mouse lemurs are an especially difficult group to study as their remains are not found in the forest due to their small size With black rats, the only previous studies to my knowledge have been performed with fecal sampling in Mandena (Ravarivolona and Ganzhorn, 2007) and with gastrointestinal dissection in Ranomafana (Lehtonen, unpubl.). While Mandena had higher diversity (14 species), Lehtonen found 5 species of intestinal nematodes in Ranomafana, which is the same number of putative species I found. The overlap in species between invasive and endemic species is limited (Fig. 8). In study I I combined my and Lehtonen's (unpubl.) samples: the only putative species which were shared between endemic and exotic species was putative species 3 which occurred in invasive *Rattus rattus* and *Mus musculus* and in endemic *Eliurus tanala*. When looking at all of my samples, the overlap is greater (Table 4, Fig. 8): Putative species (PS) 3 was shared between dogs and lemurs. Black rats and mouse lemurs shared PS1, 2 and 6. PS6 also occurred on dogs. It is noteworthy that the invasive dogs and black rats had overlapping putative species. I did not find any nematodes from *Eliurus* sp. or *Nesomys* sp., though Lehtonen had been able to find them in the same locality. As the method should have no difficulties identifying these species from fecal samples, there must have been a change in parasite community during the 12 years that separated our samplings.

The overlap in endemic species is also common. The endemic rodents share a similar putative species composition. Interesting patterns I noted was that all lemurs other than mouse lemurs (the medium-sized diurnal *Eulemur, Prolemur* and *Hapalemur*) shared the same putative species. This is not surprising, as most of the helminths in primates are able to infect several primate species (Pedersen *et al.*, 2005). This result should be considered tentative, however, as molecular methods have also uncovered new cryptic species and thus high host specificity (Anderson and Jaenike, 1997; Bouzid *et al.*, 2008). The frog species appear to have partially overlapping species composition (Harris *et al.*, in prep.), but their PS7 and PS8 are not shared by any other species (Fig. 8). This overlap is surprising as I would have expected bigger differences given that frogs have different preference for habitat: *Mantidactylus* occurs in forests while *Ptychadena* is more likely to occur in disturbed habitats, for ex-

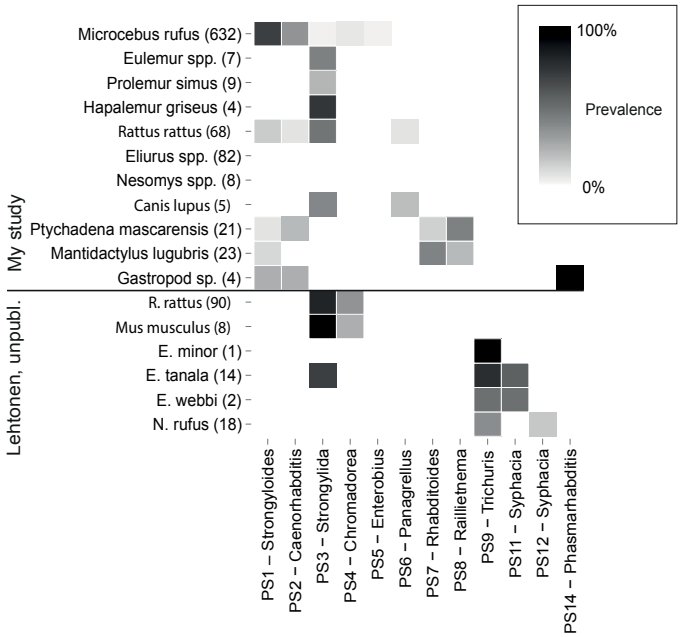


**Figure 8:** The prevalence of putative species in different host species. The overlap in putative parasite species is quite limited between endemic and invasive species. The Y-axis denotes different host species divided into two groups: the upper half of the graph shows the samples I collected and the lower half shows samples collected by Jukka T. Lehtonen. The number after each host species name is the sample size for each host. The X-axis includes the putative species and their lowest common ancestor of top scoring BLAST hits from NCBI NR database. Exotic species are written in italics. PS10 and 13 are from Finnish control samples and not included in this graph.

ample, rice fields. Nevertheless, being aquatic is the common denominator and it is possible that the similarity of aquatic environments is more important for the frog parasites than their terrestrial environment (Macrogliese *et al.*, 2005).

It appears that sympatric species potentially share the same parasite species. However, this cannot be conclusively shown as there may be insufficient resolution to distinguish between closely related species that occur in different hosts. The closely related endemic host species seemed to have similar parasite species composition, which is also an expected pattern (Poulin, 2010; Simões *et al.*, 2010; Pilosof *et al.*, 2014). In contrast, the overlap between exotic and endemic species is more limited, but the black rats and dogs shared similar species composition. Again, this was expected as invasive species often lose their own parasites when they colonize new areas (Torchin *et al.*, 2003; Prenter *et al.*, 2004) or it can be due to similar environment as invasive species occurred in only the Campsite transect.

The nematodes were clearly the most prevalent parasite group. It should be remembered that prevalence is not equivalent to the importance of parasites as the most common species are not necessarily the dominant species in the community nor do they necessarily have the highest pathogenicity or virulence (Poulin, 2007). Thus I cannot assess the importance of the most common species to the formation or succession of the community. As the sample size is rather low in some species, I have not exhaustively sampled the whole parasite community and therefore I cannot compare parasite diversity between host species.

Other parasites were studied only in mouse lemurs. The ectoparasites included only previously known species: the lice (*Lemurpediculus verruculosus*), which is known to be species-specific to *Microcebus*, and the tick (*Haemaphysalis lemuris*) (Durden *et al.*, 2010). No adult specimens of this tick species are yet found. Most probably this tick species has some of the bigger mammals as their definitive host. In both transects, the large-bodied mammals are very rare and this could explain the rareness of the ticks.

I found two closely related cestode species: *Hymenolepis* nana and *Hymenolepis diminuta*. These parasites have historically been shared by a wide range of different species, though it has been suggested that *H. nana* is a species complex with cryptic species (Macnish *et al.*, 2003). Voitto Haukisalmi (*personal communication*) has suggested a similar situation with *H. diminuta*. The lack of knowledge about these parasites is exemplified in a recent study by Barrett *et al.* (2013) where these species were merged together in a lemur health assessment, though it is plausible that different species have different effects on both lemur health and responses to environmental change. Cestodes had similar levels of prevalence (25% for *H. nana* and 26% for *H. diminuta*) and they never co-occurred in the same individuals.

The prevalence of apicomplexan coccid, *Eimeria*, was 26%. For species identification, I tried taking high quality photos of the sporulated eimeriids but the equipment in the field conditions was not sufficient and transport and storage back to Finland proved to be detrimental for the sample quality (Duszynski and Wilber, 1997). The genetic identification of eimeriids should be possible using the same pipeline I have developed, though difficulties of handling parasites, small quantities of DNA due to unicellularity and huge numbers of species might cause problems (Ogedengbe *et al.*, 2011). Mammalian eimeriids are thought to be host-specific, thus the feasibility of such studies is limited (Andrews, 1927; Kvicerová *et al.*, 2008).

## 4.3. Temporal variation of nematode infection (study III)

I succeeded in collecting and amplifying samples from 15 mouse lemurs at different time points over at least two consecutive years. This allowed me to infer the infrapopulation dynamics of the intestinal nematodes. The variation in infrapopulations was extensive. While all of the nematodes seemed to be absent from the mouse lemurs at the beginning of the season, they quickly colonized the available habitats. The lower prevalence of nematodes early in the season was most likely due to host hibernation being detrimental to the nematode community. In general, the effects of hibernation on helminths are poorly understood. Early studies were contradictory (Barnes, 1970; Coggins *et al.*, 1982), but my results can be viewed as cautiously supportive for elimination of helminths. It is possible that slightly lower numbers of parasite species in mouse lemurs compared to similarly sized rodents could be due to regular hibernation. The annual removal of nematodes could result in some of the species not being able to exist in mouse lemur, if they are too slow to colonize mouse lemurs. This is plausible as, for example, on Soay sheep the parasite levels were slow to return after anthelmintic treatment (Craig *et al.*, 2009).

In infracommunities, the most common putative species (PS1) was present almost all of the time, but the rare species were present for only short periods of time. There was no temporal trend in the presence of rare putative species. It seems that common PS1 and PS2 could also be cleared quickly as even later during the season after initial infection, they could be absent from the samples. In component communities, the most common species (PS1 and PS2) had a very stable presence whereas the rare species were ephemeral in their appearance in both communities. The method of infection might drive the stability of the most common parasites in the component community. The most abundant species, PS1, was probably Strongyloides, which has a free-living stage in soil. The free-living stage penetrates its host's skin and goes through the blood circulation and lungs to the gastrointestinal tract (Nishigori, 1928). Strongyloides is also able to autoinfect, i.e., the eggs can hatch in the intestine and the larvae penetrate through the intestinal wall to keep the infection chronic (Sandground, 1926). Putative species 2, a rhabditid nematode, could also have a similar life cycle.

The prevalence of the parasites in general seemed to be much higher in 2012 than in 2011. The prevalence was consistently high, and there was no similar increase in prevalence like was witnessed in 2011. One possible reason for this was the differing rainfall patterns.

Rainfall levels were much higher in 2012 than in 2011 throughout the dry season (Table 6). This could have led to the differences in the phenology with higher rainfall leading to higher numbers of insects and higher number of insects means there were more intermediate hosts for parasites. Furthermore, heavy rainfall could lead to hibernation being a less than optimal option for the mouse lemurs and they could wake up earlier or refrain from going into hibernation (Atsalis, 2008).

My results seem to agree with the core and satellite hypothesis of parasite metapopulations. In mouse lemurs, there were two clear core parasite species and an ever-changing group of satellite parasite species. These satellite species do not seem to be nested, they occur at and more diverse infracommunities do not have more satellite species. Nevertheless, I cannot ascertain the relative importance on these species. If the satellite species have high biomass compared to the core species, they can still exert important ecological and evolutionary pressure.

## 4.4. Parasite community in perspective (studies III-V)

The parasite community seems highly predictable. If an individual mouse lemur is caught, it probably has one or both of the common putative species. Similar stable core communities were also observed in the study on mice in Poland by Behnke *et al.* (2008b). As the prevalence of the nematodes appeared to reach almost 100% later in the field season, I would not expect substantial fitness effects for the infection, especially as the parasite presence was not detrimental to body condition (Råberg *et al.*, 2007; Jackson *et al.*, 2014; Råberg, 2014). As I was not able to assess either the fitness of the mouse lemurs or the parasite loads, this is purely speculative. However, there are also individuals that had remarkably low parasite prevalence, for example, in study III there was two individuals probably due to resistance or parasite avoidance. There were also individuals of high parasite diversity, but the parasites did not seem to be as aggregated as expected (Grenfell *et al.*, 1995; Shaw *et al.*, 1998). This would also suggest that mouse lemurs are largely tolerant to nematode infection. Nevertheless, the most heavily parasitized individuals are expected to bear the greatest fitness costs (Bordes and Morand, 2009).

This leads to an obvious big question I haven't addressed yet: what differences between individuals drive the different parasite loads or diversities? The answers to this question remain speculative at best due to the complexity of the study system (see Chapter 5). A more substantial analysis would require data from additional years. The data does allow us to look for preliminary trends and make some educated guesses. The sexes had both similarities and differences in parasite levels. The mouse lemurs are highly monomorphic as their survival, hormone levels and parasite loads seem to be similar (Zohdy *et al.*, 2014). The males had higher parasite diversity, but the sexes had the same levels of fecal egg counts and ectoparasites. I suggest the higher parasite diversity during my study is mostly linked to the shorter hibernation times in males.

Body condition (study IV) had an interesting effect: body condition, as measured by the fat reserves in the tail, was positively correlated with

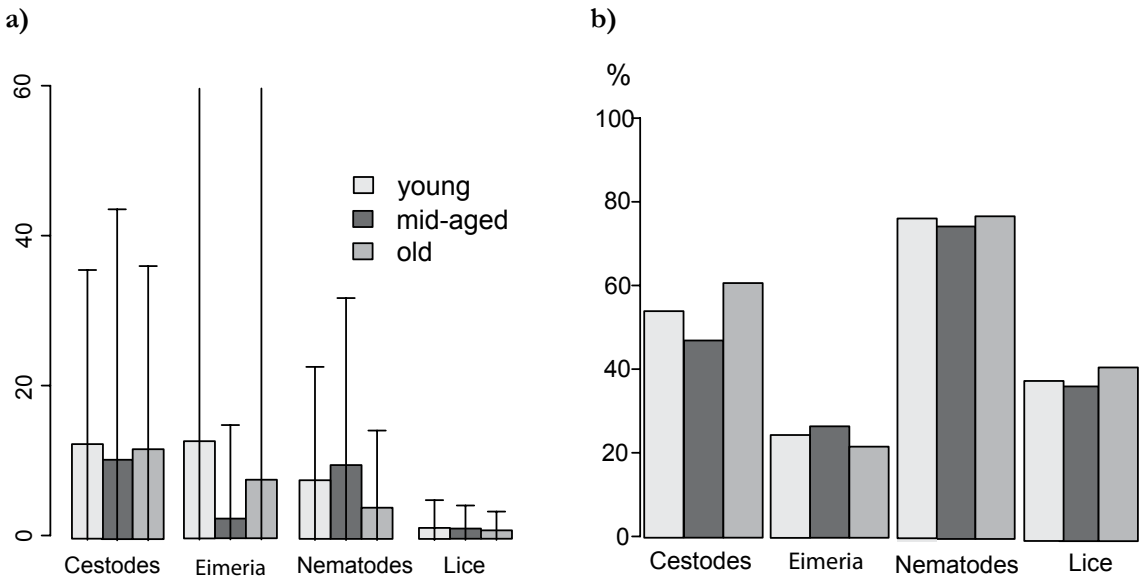


**Figure 9:** Mean parasite loads and prevalence in different age groups. The parasite loads (a) in cestodes and nematodes refer to fecal egg counts per gram feces, in *Eimeria* to spores per gram feces and in lice to total number of lice in ears. While the means may differ, the deviation is also very large. The prevalences (b) are highly similar in different age groups.

parasite richness and ectoparasite load. This implies that the mouse lemurs have the ability to resist the effects of parasitism (Råberg, 2014). The individuals in better condition might be able to sustain higher parasite load, they might move in a larger territory that could lead to more parasite encounters or the individuals might be temporarily investing in other life history traits (e.g., testosterone to increase mating success). The age did not have a clear effect in parasite loads or prevalences (Fig. 9).

The ecological context had its own effect: Campsite, the more disturbed transect, had mouse lemurs with higher prevalence of nematodes and Talatakely, the secondary forest, had mouse lemurs with higher prevalence of cestodes and especially ectoparasites. This could be explained by mouse lemur density being much higher in Talatakely: the lice are transmitted only on direct contact (Zohdy *et al.*, 2012) and cestodes are transmitted through intermediate hosts. The most common nematodes, however, are transmitted through soil contact. Multi-host parasites can also be affected by the presence of sympatric host species. Between transects there are stark differences, for example, in mammal communities: while Talatakely saw a wide range of lemur species and endemic carnivores, Campsite rarely contained any lemurs other than mouse lemurs. Semiferal dogs and invasive black rats, though, were a staple occurrence at Campsite. This also highlights the complex relationship between parasitism and habitat disturbance (Gillespie *et al.*, 2005; Chapman *et al.*, 2006; Arroyo-Rodríguez and Dias, 2010).

## 4.5. Future directions

In addition to the aforementioned results, there is a lot of potential for subsequent studies on this system. I would like to highlight some of the potential future avenues for research.

I was not able to look for intraspecific population structure of the parasites as the gene region had limited resolution. Higher resolution data would have been needed for the hibernation study: if the same strain were present before and after the dry season, this would be strong evidence for the absence of parasite turnover during hibernation. These studies would be more feasible after my study, as it is now known which putative species to target. As putative species 1 ("*Strongyloides*") was clearly the most abundant species, it would make sense to study the lower hierarchy structure of this group. Furthermore, Strongyloides commonly has multiple strain infections (Labes *et al.*, 2011), and it would be interesting to know how important intraspecific turnover is in mouse lemurs. There is also an open question as this putative species is potentially a human pathogen (Torgerson and Macpherson, 2011). Thus, additional samples from humans could reveal an interesting human-wildlife interface of zoonosis or anthroponosis (Wolfe *et al.*, 1998). The subsequent studies could also show how small-scale adaptation is possible for an abundant parasite species (Kaltz and Shykoff, 1998; Paterson and Viney, 2003).

The main problem of working with small free-living animals is the difficulty of assessing the fitness of individuals (Anderson and Gordon, 1982; Turner *et al.*, 2014). It is not therefore known what evolutionary pressures parasites are conferring on the mouse lemurs. This could only be studied by continuing long-term studies, constructing pedigrees of sampled mouse lemurs and trying to assess offspring dispersal. There is not a lot of information about the social lives or hibernation activity of rufous mouse lemurs (Atsalis, 2008). These are both central for the transmission of several pathogens. For example, microparasites are most probably transmitted in close contact, in comparison to the nematodes or cestodes that can also be transmitted via soil contact or intermediate hosts. Furthermore, studying primates is more difficult than other mammals due to the special ethical considerations attached to the handling of our close relatives (Gillespie, 2006). Thus, invasive sampling should be undertaken only to the extent required to acquire very important information. Both observational wildlife studies and experimental laboratory studies are required for the complete understanding of different factors (Hayward, 2013).

Interaction between microbiome and macroparasites in the mouse lemurs' intestine is not understood at all. Microbiome research is booming, but there have not been many studies that assess the relationship between the bacteria and the larger-sized symbionts in the host intestine. All of the studies have been done experimentally in a laboratory setting (Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Koch and Schmid-Hempel, 2012; Plieskatt *et al.*, 2013; Osborne *et al.*, 2014). This lack of studies is a definite problem as those species living in the same habitat have a huge number of direct and indirect biotic interactions (Andersen *et al.*, 2013). In fact, there is some clear evidence of helminths emulating the microbiome and indirectly affecting also the intestinal wall. I am currently following this line of inquiry as I collected fecal bacterial samples from the mouse lemurs. I expect results on this study in the near future.

There is also a sequencing project underway which could add substantial value to the already collected data. Mark Krasnow, from Stanford University, is planning to whole-genome sequence several hundred mouse lemurs to shed light on the genotype-phenotype link in wild living mammals. Potential issues which can be explored is the link between genotype and parasite resistance or tolerance, the pedigree of the mouse lemurs and its consequences for parasite spread and potential models to assess the fitness of my study individuals.

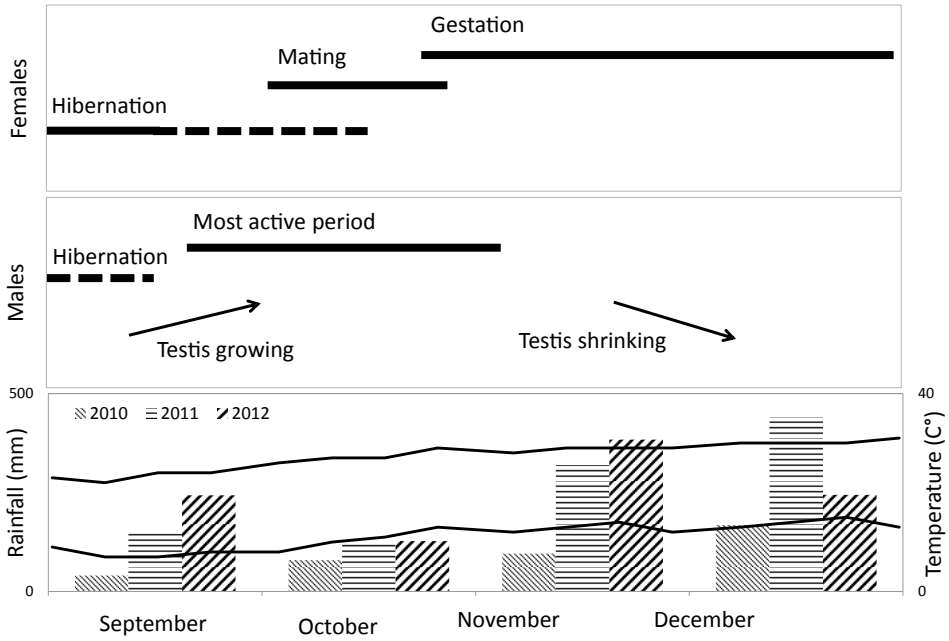


**Figure 10:** The potential drivers of parasite load and diversity in mouse lemurs. The females and males have many differential characteristics: for example females hibernate longer and they mate for only one or two nights. In comparison, males wake up earlier and their mating period is longer. Furthermore, their testes are the largest during the mating season. Age is also important characteristic, as the young individuals probably do not hibernate at all. The rainfall steadily increases during the trapping season and thus insects and fruits are more readily available later in the season. Nevertheless, rainfall is unpredictable and there are stark differences between years. In comparison, the change in temperature is less drastic and the years are very similar.

# 5. Conclusions

> ”The word ‘natural’ is meaningless. Animal strategies for feeding, reproducing or just getting about are so madly various, so utterly, gloriously perverse that you end up believing that absolutely anything is possible.”
>
> John Mitchinson & John Lloyd, *The QI Book of Animal Ignorance*

> “Only a fool does his PhD on wildlife parasites.”
>
> Juha Laakkonen, 2014

Studying wildlife parasite dynamics is inherently difficult and mouse lemurs are by no means an exception. I have collected on Figure 10 the different drivers that affect the potential parasite load and diversity during the field season I worked in Madagascar. The figure makes it clear that there is many different drivers of parasite infection on the individual host level that make it difficult to understand the importance of any individual driver. Thus I am only able to speculate the reasons for different parasite communities and loads in individuals.

Nevertheless, I managed to uncover several aspects of mouse lemurs’ and other host species’ natural intestinal parasites. I showed there is extensive turnover in the infracommunities whereas the component community remains stable. The success of the most prevalent species can be attributed to their mode of infection: a free-living stage that is able to survive for prolonged periods in the soil. The parasite prevalence seems to be modulated by the rainfall and the hibernation during the dry season. Mostly the parasite species seem to be host-specific though this requires further investigations.

The method I developed holds promise though it still needs further development. It has far-reaching potential: I successfully amplified and sequenced nematodes from very different host species, from primates to gastropods. The complete pipeline from the field to sequence analysis provides a good platform for subsequent parasitological surveys and could be also applied to other nematode studies, including soil nematodes.

# 6. Acknowledgements

No PhD thesis is an island. While this book mainly includes my blood, sweat and tears and mouse lemurs' faeces, there is a huge number of people, who have given crucial help for me. This list is going to be long, but deservedly so, for I am going to list a substantial part of people who have helped me in the past six years. I had all intentions to try and avoid regular clichés of acknowledgment section, but no, at this point it is just not possible.

First of all, my supervisors Jukka Jernvall and Juha Laakkonen were a superb duo. The first one being top academician, always knowing how to write a story and get it published, and the latter had extensive knowledge on parasites, ideas of what to do and also a care for my well-being. Busy supervisors translate to independence and freedom to pursue different projects, and I took full advantage of my freedom.

My thesis committee was as good as it can be. I am proud that Heikki Henttonen, grand old man of Finnish mammalogy and parasitology took part in it. Mar Cabeza was both critical and direct in her questions and comments - a combination which could be intimidating if it wasn't for her genuine interest – and concern – for my work and career. Mar also let me use her students of RESPECT course for data collection and additionally was pre-examiner for my thesis. Jouni Laakso was helpful in guiding me in the world of research and grant applications. He is also custos in my PhD defense.

I am honored to have Serge Morand as my opponent. I will defend my thesis only once and it is a pleasure to have a world-leading parasitologist to make sure I am fit to be PhD. I am also grateful for Eric Hoberg who was the other pre-examiner of this thesis.

Science is very collaborative project and I am not here to defend only my work, but also my contributors' work. I found Ari Löytynoja just as he was looking for real-life datasets to work with while I was getting overwhelmed by the sheer amount of sequences and my incapability to deal with them. He and Alan Medlar were more than helpful in making sense to my data. I learned many valuable lessons on working with bioinformaticians and bioinformatics. Alan has also kindly been my proof-reader on many occasions. Special thanks go to Raija Savolainen and Ritva Rice for introducing me to the world of PCR and to Agnes Viherä for invaluable help in the laboratory. Lasse, Piia and of BI sequencing facility turned feces into gold. Sarah Zohdy and Addison Kemp taught me the necessary field methods. Jani Anttila helped me substantially with statistics and R code.

I've had quite a many field assistants and may I say I've been lucky to get reliable and hard-working helpers: Herman Rafalinirina started his own PhD with mouse lemurs, Victor Rasendry was the most reliable mouse lemur handler I have met and also my closest companion in the field for three seasons. I've mentored Hannah Price from Marquette, Kelsey DeZalia from SUNY Cobleskill, Kendall Harris from Sweet Briar College, Anisha Narayan from Occidental College and Viljami Kankaanpää and Laura Sandholm from University of Helsinki.

Then there all other kinds of support PhD student can receive, going from drinking buddies to dealing with university administration and actually having something to eat.

I thank Directors of Institute of Biotechnology, Tomi Mäkelä and Howard Jacobs. Administrative and technical staff who made my life easier in Helsinki include Repe, Satu, Sanna, Minna from BI, Karin from YEB, Anni from LUOVA and Veijo from Eko-evo major.

Support from Helsinki Evo-Devo Community have been substantial. Susanna Sova was there for the most mundane needs, from "sticky" and "attaching" to booking flights and filling out travel invoices. Other members have been a verita-

ble scientific community around me - thank you Enni, Jackie, Roland, Irepan, Mona, Ian, Johann, Teemu, Outi, Mia, Rishi and Yoland. I'm also happy of the friendship I have developed with Tuomas Kankaanpää and substantial help I got with dealing biology and evolution in general.

Fellow biologists have lightened my days with too long lunch breaks and understanding what I'm ranting about when lab work didn't go as planned: Sanja, Laura, Kalle, Ulpu, Elli, Krista, Kaisa, Mikko, Martta, Alex, Emma etc.

I am happy that nowadays there is an extensive group of people who have experienced fieldwork in Madagascar. This proved to be a helpful community of people who knew what it is like to be in Madagascar. It was always pleasure to spend time in Madagascar with Titta Lassila and Susanna Kari, both also being there when it was difficult (though mostly we had fun and rum). Thanks for Tany Maitso people, Suomi-Madagascar society and Manondroala project of Finnish Association of Nature Conservation (Hilkka, Kaisa, Tiina, Tanjona, Olli). Special mention goes to Jukka T Lehtonen, who could fit many categories of people I need to thank: he arranged with Mar the first ever RESPECT course in Centre Valbio in 2008. If it were not for that course, I would not have chosen this subject for my thesis. I got also use of Jukka's samples for the publications in this thesis.

Centre Valbio staff eased my sejour in the field: Eileen, John, Pascal, Jean-Claude, Dede, Jean de Dieu, Mamisoa and especially the able and kind personnel of kitchen (misaotra Madame Angele!), cleaning personnel and guards. Pain of separating from my loved ones in Finland for extended fieldwork was always lessened when I heard the friendly "tonga soa".

Expatriate community in Centre Valbio is vast. Silvija Budaviciute was doing her PhD at the same time with me and helped with various practical things. Sylvia Atsalis provided me with a lot of professional advice and emotional support. Patricia Wright was almost my supervisor-in-field with continual ideas, suggestions and encouragement. Rachel Jacobs is the sweetest person I know, (and of the all people, she deserves that her lab work goes smoothly). Also thanks for the company goes to Tharcisse, Ingrid, James, Amaranta, Ricardo, Riikka, Aurora, Akvile, Bashira, Sam and many other people staying for shorter periods in Valbio. I am also in gratitude to all those Malagasy people who have helped during the 12 months I've spent in that beautiful country. Misaotra betsaka!

Malagasy authorities gave me the permits to work in Grande Ile Rouge: Ministry of Water and Forests, Madagascar National Parks and Tri-partite Comission for Research and MICET facilitated my field work. Special thanks go to Ranomafana National Park manager Mamy Rakotoarijaona and Benjamin Andriamahaja from MICET who have helped me with problems in Malagasy bureaucracy. My work was also accepted by the committee of ethical assessment of research in Viikki campus, University of Helsinki.

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I had an impressive, but never really exhausting, number of side projects during my PhD studies. Just to mention the most important ones: I found my inner science popularizer and communicator and I'm happy to have the occasion to write, speak and feature my research in many outlets, ranging from French TV5 to Radio Nova. I've worked with many people in University PR and press unit and have had the chance to have my very own blog at Tiede magazine. For these opportunities I'm very grateful.

I also happened to do second master thesis, this time in biology education: Anna Uitto was my supervisor and Mauri Åhlberg gave invaluable help on my quest to save Finnish genetics

teaching in high school-level. Even more, I'm happy to have been given the chance to influence the national curriculum development process in biology education for both primary and upper secondary schools. I had an amazing opportunity to participate in International Biology Olympiad as an Jury member, plan the national competition and organize the training of Finnish team. IBO team, Matias, Pinja, Eira, Tiina, Justus and all wonderful teachers in training course, were a wonderfully enjoyable group of people.

All mentioned above have really known what I have been doing. Then there is also all those friends who did not have a clue (besides seeing photos of cute mouse lemurs) what I was ranting about. They have been understanding and ever helpful in downing a glass, or two, of wine when the situation required it. So, here we go: Maria, Arttu, Mikko, Marsa, Silja, Mirva, Mirja, Ville and the list goes on. Both my wives, Lotta Aarikka and Riikka Kinnunen, always deserve a special mention. My family was always supportive, even my mother who eventually got used to my constant travel to that dangerous place (and have been providing me with never-ending supply of technical shirts). Like I wrote in the beginning, this is a long list and we're not in the end yet. It just proves the point that science nowadays is not about individual achievement, but a fundamentally social undertaking.

I started this list with the most important people for this dissertation and I am going to end it with the most important person, though for the very different reason. My husband Teemu Leminen has given me support in most imaginable ways and continuous to do so. Our relationship started around the same time I started courting my research topic and both of these relationships have now entered their next stage. It has been a wonderful experience to find a real soulmate, who doesn't really know what I'm doing but understands that whenever I mention PCR, it is a good idea to pour a glass, or two, of wine.

It's almost seven years ago when I met Jukka in Centre Valbio and this project was started. Nothing suits better for this occasion, than words of Pentti Saarikoski (from the prologue of his translation of Ulysseus):

"*Työ on tehty ja vaiva on nähty, ja nyt aion syödä ja juoda jumalaiseen aamunkoittoon asti.*"

# 7. References


**Abebe, E., Mekete, T. and Thomas, W. K.** (2011). A critique of current methods in nematode taxonomy. *African Journal of Biotechnology* **10**, 312–323.

**Agosta, S. J., and Klemens, J.A. (**2008). Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecology Letters* **11**, 1123-1134.

**Alizon, S.** (2013a). Co-infection and super-infection models in evolutionary epidemiology. *Interface Focus* **3**, 20130031.

**Alizon, S.** (2013b). Parasite co-transmission and the evolutionary epidemiology of virulence. *Evolution* **67**, 921–933.

**Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M. and Rohani, P.** (2006). Seasonality and the dynamics of infectious diseases. *Ecology Letters* **9**, 467–84.

**Andersen, L. O., Vedel Nielsen, H. and Stensvold, C. R.** (2013). Waiting for the human intestinal Eukaryotome. *The ISME journal* 1–3. doi:10.1038/ismej.2013.21.

**Anderson, R. M. and Gordon, D. M.** (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

**Anderson, T. J. C. and Jaenike, J.** (1997). Host specificity , evolutionary relationships and macrogeographic differentiation among Ascaris populations from humans and pigs. *Parasitology* **115**, 325–342.

**Andrews, J.** (1927). Host-parasite specificity in the Coccidia of mammals. *The Journal of Parasitology* **13**, 183–194.

**Arneberg, P., Skorping, A., Grenfell, B. and Read, A. F.** (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B: Biological Sciences* **265**, 1283–1289.

**Arroyo-Rodríguez, V. and Dias, P. A. D.** (2010). Effects of habitat fragmentation and disturbance

on howler monkeys: a review. *American Journal of Primatology* **72**, 1–16.

**Asmundsson, I.M., Mortenson, J.A., and Hoberg, E.P.** (2008). Muscleworms, *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae), discovered in Columbia white-tailed deer from Oregon and Washington: Implications for biogeography and host associations. *Journal of Wildlife Diseases*. **44**, 16-27.

**Atsalis, S.** (1999). Seasonal fluctuations in body fat and activity levels in a rainforest species of moue lemur (Microcebus rufus). *International Journal of Primatology* **20**, 883–910.

**Atsalis, S.** (2008). *A natural history of the brown mouse lemur*. Pearson Education, Upper Saddle River, NJ, USA.

**Barnes, E. M.** (1970). Effect of hibernation intestinal flora. *The American Journal of Clinical Nutrition* **23**, 1519–1524.

**Barrett, M. a., Brown, J. L., Junge, R. E. and Yoder, A. D.** (2013). Climate change, predictive modeling and lemur health: Assessing impacts of changing climate on health and conservation in Madagascar. *Biological Conservation* **157**, 409–422.

**Bazzone, L. E., Smith, P. M., Rutitzky, L. I., Shainheit, M. G., Urban, J. F., Setiawan, T., … Stadecker, M. J.** (2008). Coinfection with the intestinal nematode Heligmosomoides polygyrus markedly reduces hepatic egg-induced immunopathology and proinflammatory cytokines in mouse models of severe schistosomiasis. *Infection and Immunity* **76**, 5164–5172.

**Behnke, J. M.** (2008). Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions .... or pure randomness? *Parasitology* **135**, 751–766.

**Behnke, J. M., Gilbert, F. S., Abu-Madi, M. a. and Lewis, J. W.** (2005). Do the helminth parasites of wood mice interact? *Journal of Animal Ecology* **74**, 982–993.

**Behnke, J. M., Bajer, A., Harris, P. D., Newington, L., Pidgeon, E., Rowlands, G., … Barnard, C. J.** (2008a). Temporal and between-site variation in helminth communities of bank voles (Myodes glareolus) from N.E. Poland. 1. Regional fauna and component community levels. *Parasitology* **135**, 985–997.

**Behnke, J. M., Bajer, A., Harris, P. D., Newington, L., Pidgeon, E., Rowlands, G.,… Barnard, C. J.** (2008b). Temporal and between-site variation in helminth communities of bank voles (Myodes glareolus) from N.E. Poland. 2. The infracommunity level. *Parasitology* **135**, 999–1018.

**Beldomenico, P. M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., & Begon, M.** (2008). Poor condition and infection: a vicious circle in natural populations. *Proceedings of the Royal Society B: Biological Sciences* **275**, 1753–9.

**Beldomenico, P. M. and Begon, M.** (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology & Evolution* **25**, 21–7.

**Benavides, J. A, Huchard, E., Pettorelli, N., King, A. J., Brown, M. E., Archer, C. E., … Cowlishaw, G.** (2012). From parasite encounter to infection: multiple-scale drivers of parasite richness in a wild social primate population. *American Journal of Physical Anthropology* **147**, 52–63.

**Beraldi, D., McRae, A. F., Gratten, J., Pilkington, J. G., Slate, J., Visscher, P. M. and Pemberton, J. M.** (2007). Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (Ovis aries). *International Journal for Parasitology* **37**, 121–9.

**Bik, H. M., Porazinska, D. L., Creer, S., Caporaso, J. G., Knight, R. and Thomas, W. K.** (2012). Sequencing our way towards understanding global eukaryotic biodiversity. *Trends in Ecology & Evolution* **27**, 233–43.

**Blanco, M. B.** (2008). Reproductive schedules of female Microcebus rufus at Ranomafana National Park, Madagascar. *International Journal of Primatology* **29**, 323–338.

**Blanco, M. B.** (2011). Timely estrus in wild brown mouse lemur females at Ranomafana National Park, southeastern Madagascar. *American Journal of Physical Anthropology* **145**, 311–317.

**Blaxter, M. L.** (2004). The promise of a DNA taxonomy. *Philosophical transactions of the Royal Society of London B: Biological sciences* **359**, 669–79.

**Bochkov, A. V., Klimov, P. B., & Wauthy, G. (2011).** Phylogeny and coevolutionary associations of makialgine mites (Acari, Psoroptidae, Makialginae) provide insight into evolutionary history of their hosts, strepsirrhine primates. *Zoological Journal of the Linnean Society* **162,** 1–14.

**Bordes, F. and Morand, S.** (2009). Coevolution between multiple helminth infestations and basal immune investment in mammals: cumulative effects of polyparasitism? *Parasitology Research* **106**, 33–7.

**Bordes, F. and Morand, S.** (2011). The impact of multiple infections on wild animal hosts: a review. *Infection Ecology & Epidemiology* **1**, 7346.

**Bouzid, W., Stefka, J., Hypsa, V., Lek, S., Scholz, T., Legal, L., Ben Hassine, O. K. and Loot, G.** (2008). Geography and host specificity: two forces behind the genetic structure of the freshwater fish parasite Ligula intestinalis (Cestoda: Diphyllobothriidae). *International Journal for Parasitology* **38**, 1465–79.

**Breuil, J., and Coulanges, P.** (1982) Note sure *Angiostrongylus cantonensis* à Madagascar. *Archives de l'Institute Pasteur de Madagascar* **50**, 35-8.

**Broadhurst, M. J., Ardeshir, A., Kanwar, B., Mirpuri, J., Gundra, U. M., Leung, J. M., … Loke, P.** (2012). Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon. *PLoS Pathogens* **8**, e1003000.

**Brooks, D.R., Hoberg, E.P., Gardner, S.L., Boeger, W., Galbreath, K.E., Herczeg, D., Mejía-Madrid, H.H., Racz, E. and Tsogtsaikhan, A**. (2014). Finding them before they find us: Informatics, parasites and environments in accelerating climate change. *Comparative Parasitology* **81**, 155-164.

**Brown, E. A., Pilkington, J. G., Nussey, D. H., Watt, K. A., Hayward, A. D., Tucker, R., … Slate, J.** (2013). Detecting genes for variation in parasite burden and immunological traits in a wild population: testing the candidate gene approach. *Molecular Ecology* **22**, 757–73.

**Budischak, S. a, Hoberg, E. P., Abrams, A., Jolles, A. E., & Ezenwa, V. O.** (2015). A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. *Molecular Ecology Resources,* **in press.**

**Bush, A. O. and Lotz, J. M.** (2000). The ecology of "crowding." *Journal of Parasitology* **86**, 212–213.

**Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W.** (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *The Journal of parasitology* **83**, 575–583.

**Bush, A. O., Caira, J. N., Minchella, D. J., Nadler, S. A. and Seed, J. R.** (2001). *Parasitism: The diversity and ecology of animal parasites*. Cambridge University Press, Cambridge, UK.

**Cardon, M., Loot, G., Grenouillet, G. and Blanchet, S.** (2011). Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: a multilevel causal analysis. *The Journal of Animal Ecology* **80**, 657–67.

**Carleton, M. D.** (2003). Eliurus: tufted-tailed rats. In *The Natural History of Madagascar* (ed. Goodman, S. M. and Benstead, J. P.), pp. 1373–1380. University of Chicago Press, Chicago, IL, USA.

**Caron, D. A.** (2009). New Accomplishments and Approaches for Assessing Protistan Diversity and Ecology in Natural Ecosystems. *BioScience* **59**, 287–299.

**Carvalho, M. R., Bockmann, F.A., Amorim, D. S., Brandão, C. R. F., Vivo, M., Figueiredo, J. L., … Nelson, G. J.** (2007). Taxonomic impediment or impediment to taxonomy? A commentary on systematics and the cybertaxonomic-automation paradigm. *Evolutionary Biology* **34**, 140–143.

**Casiraghi, M., Labra, M., Ferri, E., Galimberti, A. and De Mattia, F.** (2010). DNA barcoding: a six-question tour to improve users' awareness about the method. *Briefings in Bioinformatics* **11**, 440–53.

**Cattadori, I. M., Albert, R. and Boag, B.** (2007). Variation in host susceptibility and infectiousness generated by co-infection: the myxoma-Trichostrongylus retortaeformis case in wild rabbits. *Journal of the Royal Society Interface* **4**, 831–40.

**Chabaud, A. G., Brygoo, E.-R. and Petter, A.-J**. (1965). Les Nématodes parasites de Lémuriens malgaches VI. Description de six espèces nouvelles et conclusions générales. *Annales de Parasitologie Humaine et Comparée,* **40**, 181-214.

**Chapman, C. A., Speirs, M. L., Gillespie, T. R., Holland, T. and Austad, K. M.** (2006). Life on the edge : gastrointestinal parasites from the forest edge and interior primate groups. *American Journal of Primatology* **68**, 397–409.

**Clough, D.** (2010). Gastro-intestinal parasites of red-fronted lemurs in Kirindy Forest, western Madagascar. *The Journal of Parasitology* **96**, 245–251.

**Clough, D., Heistermann, M. and Kappeler, P. M.** (2010). Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (Eulemur fulvus rufus). *American Journal of Physical Anthropology* **142**, 441–52.

**Coggins, J. R., Tedesco, J. L. and Rupprecht, C. E.** (1982). Seasonal changes and overwintering of parasites in the bat, Myotis lucifugus (Le Conte), in a Wisconsin Hibernaculum. *American Midland Naturalist* **107**, 305–315.

**Cooper, N., Kamilar, J. M. and Nunn, C. L.** (2012). Host longevity and parasite species richness in mammals. *PloS One* **7**, e42190.

**Craig, B. H., Jones, O. R., Pilkington, J. G. and Pemberton, J. M.** (2009). Re-establishment of nematode infra-community and host survivorship in wild Soay sheep following anthelmintic treatment. *Veterinary Parasitology* **161**, 47–52.

**Creer, S., Fonseca, V. G., Porazinska, D. L., Giblin-Davis, R. M., Sung, W., Power, D. M., … Thomas, W. K.** (2010). Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology* **19 Suppl 1**, 4–20.

**Dallas, T. and Presley, S. J.** (2014). Relative importance of host environment, transmission potential and host phylogeny to the structure of parasite metacommunities. *Oikos* **123**, 866–874.

**Davies, T. J. and Pedersen, A. B.** (2008). Phylogeny

and geography predict pathogen community similarity in wild primates and humans. *Proceedings of the Royal Society B* **275**, 1695–1701.

**Deagle, B. E., Kirkwood, R. and Jarman, S. N.** (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology* **18**, 2022–38.

**DeSalle, R., Egan, M. G. and Siddall, M.** (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society of London B: Biological sciences* **360**, 1905–16.

**Dobson, A.** (1985). The population dynamics of competition between parasites. *Parasitology* **91**, 317–347.

**Dobson, A., Lafferty, K. D., Kuris, A. M., Hechinger, R. F. and Jetz, W.** (2008). Homage to Linnaeus: How many parasites ? How many hosts ? *Proceedings of National Academies of Sciences* **105**, 11482–11489.

**Dorchies, P., Bergeaud, J. P., Khanh, N. Van and Morand, S.** (1997). Reduced egg counts in mixed infections with Oestrus ovis and Haemonchus contortus: influence of eosinophils? *Parasitology Research* **83**, 727–730.

**Durden, L. A., Zohdy, S. and Laakkonen, J.** (2010). Lice and ticks of the eastern rufous mouse lemur, Microcebus rufus, with descriptions of the male and third instar nymph of Lemurpediculus verruculosus (Phthiraptera: Anoplura). *The Journal of Parasitology* **96**, 874–878.

**Durette-Desset, M.-C., Lehtonen, J., and Haukisalmi, V.** (2002). *Trichostrongylina* (Nematoda) from Malagasy Muridae I - Description of two new species of Heligmonellidae in *Nesomys* spp. *Parasite* **9**, 127-33.

**Duszynski, D. W. and Wilber, P. G.** (1997). A guideline for the preparation of species descriptions in the Eimeridaee. *The Journal of Parasitology* **83**, 333–336.

**Ebach, M. C. and Holdrege, C.** (2005). More taxonomy, not DNA barcoding. *BioScience* **55**, 822–823.

**Ebert, D., Hottinger, J. W. and Pajunen, V. I.** (2001). Temporal and spatial dynamics of parasite richness in a Daphnia metapopulation. *Ecology* **82**, 3417–3434.

**Emerson, B. C., Cicconardi, F., Fanciulli, P. P. and Shaw, P. J. A.** (2011). Phylogeny, phylogeography, phylobetadiversity and the molecular analysis of biological communities. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**, 2391–2402.

**Eysker, M. and Ploeger, H. W.** (2000). Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. *Parasitology* **120**, S109–S119.

**Ezenwa, V. O. and Jolles, A. E.** (2011). From host immunity to pathogen invasion: the effects of helminth coinfection on the dynamics of microparasites. *Integrative and Comparative Biology* **51**, 540–51.

**Fedorka, K. M., Zuk, M. and Mousseau, T. A.** (2004). Immune suppression and the cost of reproduction in the ground cricket, Allonemobius socius. *Evolution* **58**, 2478–2485.

**Fenton, A., Viney, M. E. and Lello, J.** (2010). Detecting interspecific macroparasite interactions from ecological data: patterns and process. *Ecology letters* **13**, 606–15.

**Ferri, E., Barbuto, M., Bain, O., Galimberti, A., Uni, S., Guerrero, R., … Casiraghi, M.** (2009). Integrated taxonomy: traditional approach and DNA barcoding for the identification of filarioid worms and related parasites (Nematoda). *Frontiers in Zoology* **6**, 1.

**Ferris, H., Griffiths, B. S., Porazinska, D. L., Powers, T. O., Wang, K.-H. and Tenuta, M.** (2012). Reflections on plant and soil nematode ecology: past, present and future. *Journal of Nematology* **44**, 115–26.

**Floyd, R., Abebe, E., Papert, A. and Blaxter, M.** (2002). Molecular barcodes for soil nematode identification. *Molecular Ecology* **11**, 839–50.

**Font, W. F.** (1998). Parasites in paradise: patterns of helminth distribution in Hawaiian stream fishes. *Journal of Helminthology* **72**, 307–311.

**Frézal, L. and Leblois, R.** (2008). Four years of DNA barcoding: current advances and prospects. *Infection, Genetics and Evolution* **8**, 727–736.

**Garbutt, N.** (2007). *Mammals of Madagascar: A Complete Guide*. Yale University Press, New Haven, CO, USA.

**Gardner, M. P., Gems, D., & Viney, M. E. (2006).** Extraordinary plasticity in aging in Strongyloides ratti implies a gene-regulatory mechanism of lifespan evolution. *Aging Cell* **5**, 315–23.

**Gasser, R. B.** (2006). Molecular tools - advances, opportunities and prospects. *Veterinary Parasitology* **136**, 69–89.

**Gasser, R. B., Bott, N. J., Chilton, N. B., Hunt, P. and Beveridge, I.** (2008). Toward practical, DNA-based diagnostic methods for parasitic nematodes of livestock--bionomic and biotechnological implications. *Biotechnology Advances* **26**, 325–34.

**Gaugler, R., Lewis, E. and Stuart, R. J.** (1997). Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia* **109**, 483–489.

**George-Nascimento, M., Muñoz, G., Marquet, P. a. and Poulin, R.** (2004). Testing the energetic equivalence rule with helminth endoparasites of

vertebrates. *Ecology Letters* **7**, 527–531.

**Gillespie, T. R.** (2006). Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology* **27**, 1129–1143.

**Gillespie, T. R., Chapman, C. A. and Greiner, E. C.** (2005). Effects of logging on gastrointestinal parasite infections and infection risk in African primates. *Journal of Applied Ecology* **42**, 699–707.

**Godfray, H. C. J.** (1994). *Parasitoids: behavioral and evolutionary ecology*. Princeton University Press, Princeton, NJ, USA.

**Goldstein, P. Z. and DeSalle, R.** (2011). Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. *BioEssays* **33**, 135–47.

**Gómez, A. and Nichols, E.** (2013). Neglected wild life: Parasitic biodiversity as a conservation target. *International Journal for Parasitology. Parasites and Wildlife* **2**, 222–227.

**Gotelli, N. J. and Colwell, R. K.** (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* **4**, 379–391.

**Gotelli, N. J. and Ulrich, W.** (2012). Statistical challenges in null model analysis. *Oikos* **121**, 171–180.

**Gourbière, S., Morand, S. and Waxman, D.** (2015). Fundamental factors determining the nature of parasite aggregation in hosts. *PloS one* **10**, e0116893.

**Gregory, R. D., Keymer, A. E. and H, H. P.** (1996). Helminth parasite richness among vertebrates. *Biodiversity and Conservation* **5**, 985–997.

**Greischar, M. and Koskella, B.** (2007). A synthesis of experimental work on parasite local adaptation. *Ecology letters* **10**, 418–34.

**Grenfell, B. T., Wilson, K., Isham, V. S., Boyd, H. E. and Dietz, K.** (1995). Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode-ruminant interactions as a case study. *Parasitology* **111 Suppl**, S135–51.

**Griffin, R. H. and Nunn, C. L.** (2011). Community structure and the spread of infectious disease in primate social networks. *Evolutionary Ecology* **26**, 779–800.

**Guégan, J.-F. and Kennedy, C. R.** (1993). Maximum local helminth parasite community richness in British freshwater fish: a test of the colonization time hypothesis. *Parasitology* **106**, 91–100.

**Guégan, J.-F., Morand, S. and Poulin, R.** (2005). Are there general laws in parasite community ecology? The emergence of spatial parasitology and epidemiology. In *Parasitism and Ecosystems* (ed. Thomas, F., Renaud, F., and Guégan, J.-F.), p. 229. Oxford University Press, Oxford, UK.

**Hamilton, W. D.** (1980). Sex versus non-sex versus parasite. *Oikos* **35**, 282–290.

**Hatcher, M., Dick, J. T. A. and Dunn, A. M.** (2012). Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment* **10**, 186–194.

**Haukisalmi, V. and Henttonen, H.** (1990). The impact of climatic factors and host density on the long-term population dynamics of vole helminths. *Oecologia* **83**, 309–315.

**Haukisalmi, V. and Henttonen, H.** (1998). Analysing interspecific associations in parasites: alternative methods and effects of sampling heterogeneity. *Oecologia* **116**, 565–574.

**Haukisalmi, V., Henttonen, H. and Tenora, F.** (1988). Population dynamcis of common and rare helminths in cyclic vole populations. *Journal of Animal Ecology* **57**, 807–825.

**Haukisalmi, V., Heino, M. and Kaitala, V.** (1998). Body size variation in tapeworms (Cestoda): adaptation to intestinal gradients? *Oikos* **83**, 152–160.

**Hawley, D. M. and Altizer, S. M.** (2011). Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology* **25**, 48–60.

**Hayward, A. D.** (2013). Causes and consequences of intra- and inter-host heterogeneity in defence against nematodes. *Parasite Immunology* 362–373.

**Hayward, A. D., Wilson, A. J., Pilkington, J. G., Pemberton, J. M. and Kruuk, L. E. B.** (2009). Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3477–85.

**Hayward, A. D., Nussey, D. H., Wilson, A. J., Berenos, C., Pilkington, J. G., Watt, K. A, … Graham, A. L.** (2014). Natural selection on individual variation in tolerance of gastrointestinal nematode infection. *PLoS Biology* **12**, e1001917.

**Hebert, P. D. N., Cywinska, A., Ball, S. L. and DeWaard, J. R.** (2003). Biological identifications through DNA barcodes. *Proceedings. Biological sciences / The Royal Society* **270**, 313–21.

**Hendry, A. P., & Gonzalez, A.** (2008). Whither adaptation? *Biology & Philosophy* **23**, 673–699.

**Herrera, J. P., Wright, P. C., Lauterbur, E., Ratovonjanahary, L. and Taylor, L. L.** (2011). The effects of habitat disturbance on lemurs at Ranomafana National Park, Madagascar. *International Journal of Primatology* **32**, 1091–1108.

**Hingston, M., Goodman, S. M., Ganzhorn, J.**

**U. and Sommer, S.** (2005). Reconstruction of the colonization of southern Madagascar by introduced Rattus rattus. *Journal of Biogeography* **32**, 1549–1559.

**Hoberg, E.P.** (2010) Invasive processes, mosaics and the structure of helminth parasite faunas. *Revue Scientifique et Technique Office International des Épizooties* **29**, 255-272.

**Hoberg E.P., Agosta, S.J., Boeger, W.A., and Brooks, D.R..** (2015). An Integrated parasitology: Revealing the elephant through tradition and invention. *Trends in Parasitology* **31** 128-133.

**Hoberg. E. P., and Brooks, D. R.** (2008). A macroevolutionary mosaic: Episodic host-switching, geographic colonization, and diversification in complex host-parasite systems. *Journal of Biogeography* **35,** 1533-1550.

**Hoberg, E.P. and Brooks, D.R.** (2015). Evolution in action: Climate change, biodiversity dynamics and emerging infectious disease. Theme Issue- Climate Change and Vector-Borne Diseases. *Philosophical Transactions of the Royal Society B*: **370**, 20130553.

**Hoberg, E.P., Galbreath, K.E., Cook, J.A., Kutz, S.J. and Polley, L**. (2012). Northern host-parasite assemblages: History and biogeography on the borderlands of episodic climate and environmental transition. D. Rollinson and S.I. Hays (eds.). Elsevier. *Advances in Parasitology*. **79**, 1-97.

**Huang, S., Bininda-Emonds, O. R. P., Stephens, P. R., Gittleman, J. L. and Altizer, S.** (2013). Phylogenetically related and ecologically similar carnivores harbour similar parasite assemblages. *Journal of Animal Ecology* **83**, 671–680.

**Hudson, M. E.** (2008). Sequencing breakthroughs for genomic ecology and evolutionary biology. *Molecular Ecology Resources* **8**, 3–17.

**Irwin, M. T. and Raharison, J. L.** (2009). A review of the endoparasites of the lemurs of Madagascar. *Malagasy Nature* **2**, 66–93.

**Jackson, J. A., Hall, A. J., Friberg, I. M., Ralli, C., Lowe, A., Zawadzka, M., … Begon, M.** (2014). An immunological marker of tolerance to infection in wild rodents. *PLoS Biology* **12**, e1001901.

**Jarman, S. N., Deagle, B. E. and Gales, N. J.** (2004). Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. *Molecular Ecology* **13**, 1313–22.

**Jenkins, E.J., Appleyard, G.D., Hoberg, E.P., Rosenthal, B.M., Kutz, S.J., Veitch, A, … Polley L.** (2005). Geographic distribution of the muscle dwelling nematode *Parelaphostrongylus odocoilei* (Protostrongylidae: Elaphostrongylinae) in North America using molecular identification of first-stage larvae. *Journal of Parasitology* **91**, 574-584.

**Johnson, P. T. J. and Hoverman, J. T.** (2012). Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 9006–11.

**Johnson, P. T. J. and Thieltges, D. W.** (2010). Diversity, decoys and the dilution effect: how ecological communities affect disease risk. *The Journal of Experimental Biology* **213**, 961–70.

**Jolles, A. E., Ezenwa, V., Etienne, R. S., Turner, W. C. and Olff, H.** (2008). Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. *Ecology* **89**, 2239–2250.

**Joly, M. and Zimmermann, E.** (2011). Do solitary foraging nocturnal mammals plan their routes? *Biology Letters* **7**, 638–40.

**Jorge, F., Carretero, M. A, Roca, V., Poulin, R. and Perera, A.** (2013). What you get is what they have? Detectability of intestinal parasites in reptiles using faeces. *Parasitology Research* **112**, 4001–4007.

**Joyeux, C.E. and Baer, G.** (1936). Helminthes des rats de MAdagascar: contribution à l'étude de *Davainea madagascarensis*. *Bulletin de la Société de Pathologie Exotique et de ses Filiales* **29**, 611-19.

**Kaltz, O. and Shykoff, J. A.** (1998). Local adaptation in host-parasite systems. *Heredity* **81**, 361–370.

**Kamiya, T., O'Dwyer, K., Nakagawa, S. and Poulin, R.** (2014). What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. *Biological Reviews of the Cambridge Philosophical Society* **89**, 123–34.

**Kennedy, C. R. and Guégan, J.-F.** (1996). The number of niches in intestinal helminth communities of Anguilla anguilla: are there enough spaces for parasites? *Parasitology* **113**, 293–302.

**Keymer, A.** (1982). Density-dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology* **84**, 573–587.

**King, R. A., Read, D. S., Traugott, M. and Symondson, W. O. C.** (2008). Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology* **17**, 947–63.

**Knowles, S. C. L., Fenton, A., Petchey, O. L., Jones, T. R., Barber, R. and Pedersen, A. B.** (2013). Stability of within-host – parasite communities in a wild mammal system. *Proceedings of the Royal Society B: Biological Sciences* **280**,. doi:10.1098/rspb.2013.0598.

**Kobbe, S. and Dausmann, K. H.** (2009). Hibernation in Malagasy mouse lemurs as a strategy to counter environmental challenge. *Die Naturwissenschaften* **96**, 1221–7. doi:10.1007/s00114-009-0580-3.

**Koch, H. and Schmid-Hempel, P.** (2012). Gut

microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. *Ecology Letters* **15**, 1095–103.

**Kutz, S.J., Checkley, S., Verocai, G.G., Dumond, M., Hoberg, E.P, Peacock, R., … Abrams, A.** (2013). Invasion, establishment, and range expansion of two parasitic nematodes in the Canadian Arctic. *Global Change Biology* **19** 3254-3262.

**Kvicerová, J., Pakandl, M. and Hypsa, V.** (2008). Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. *Parasitology* **135**, 443–52.

**Laakkonen, J., Lehtonen, J. T., & Wright, P. C.** (2003). Trypanosome parasites in the invading Rattus rattus and endemic rodents in Madagascar. In G. R. Singleton, L. A. Hinds, C. Krebs, & S. Dave (Eds.), **Rats, mice and people : rodent biology and management** (pp. 37–40). Canberra, Australia: Australian Centre for International Agricultural Research.

**Labes, E. M., Wijayanti, N., Deplazes, P. and Mathis, A.** (2011). Genetic characterization of *Strongyloides* spp. from captive, semi-captive and wild Bornean orangutans (Pongo pygmaeus) in Central and East Kalimantan, Borneo, Indonesia. *Parasitology* **138**, 1417–22.

**Lajeunesse, M. J. and Forbes, M. R.** (2002). Host range and local parasite adaptation. *Proceedings of the Royal Society B: Biological Sciences* **269**, 703–10.

**Lee, M. S. Y.** (2004). The molecularisation of taxonomy. *Invertebrate Systematics* **18**, 1–6.

**Lee, K. A.** (2006). Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology* **46**, 1000–15.

**Lehtonen, J.T.** (2013) *Occurrence of the introduced black rat (Rattus rattus) and its potential effects on endemic rodents in Southeastern Madagascar.* PhD thesis, University of Helsinki.

**Lehtonen, J. T., Mustonen, O., Ramiarinjanahary, H., Niemelä, J. and Rita, H.** (2001). Habitat use by endemic and introduced rodents along a gradient of forest disturbance in Madagascar. *Biodiversity and Conservation* **10**, 1185–1202.

**Lello, J., Norman, R. a, Boag, B., Hudson, P. J. and Fenton, A.** (2008). Pathogen interactions, population cycles, and phase shifts. *The American Naturalist* **171**, 176–82.

**Leung, T. L. F. and Poulin, R.** (2008). Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu - Life and Environment* **58**, 107–115.

**Leung, T. L. F., Donald, K. M., Keeney, D. B., Koehler, A. V, Peoples, R. C. and Poulin, R.** (2009). Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: life cycles , ecological roles and DNA barcodes. *New Zealand Journal of Marine and Freshwater Research* **43**, 857–865.

**Lion, S. and Gandon, S.** (2014). Evolution of spatially structured host-parasite interactions. *Journal of Evolutionary Biology* **28**, 10–28.

**Locke, S. a, McLaughlin, J. D., Lapierre, A. R., Johnson, P. T. J. and Marcogliese, D. J.** (2011). Linking larvae and adults of Apharyngostrigea cornu, Hysteromorpha triloba, and Alaria mustelae (Diplostomoidea: Digenea) using molecular data. *Journal of Parasitology* **97**, 846–51.

**Loot, A. G., Blanchet, S., Aldana, M., & Navarrete, S. A.** (2008). Evidence of plasticity in the reproduction of a trematode parasite: the effect of host removal. *Journal of Parasitology* **94** 23–27.

**Macnish, M. G., Morgan-Ryan, U. M., Monis, P. T., Behnke, J. M. and Thompson, R. C. a.** (2003). A molecular phylogeny of nuclear and mitochondrial sequences in *Hymenolepis nana* (Cestoda) supports the existence of a cryptic species. *Parasitology* **125**, 567–575.

**Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M. V and Welburn, S. C.** (2013). A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution and risk factors. *Parasites & Vectors* **6**, 239.

**Marcogliese, D. J.** (1997). Fecundity of sealworm (*Pseudoterranova decipien*s) infecting grey seals (Halichoerus grypus) in the Gulf of St . Lawrence, Canada: lack of density-dependent effects. *International Journal for Parasitology* **27**, 1401–1409.

**Marr, S. R., Mautz, W. J. and Hara, A. H.** (2007). Parasite loss and introduced species: a comparison of the parasites of the Puerto Rican tree frog, (*Eleutherodactylus coqui*), in its native and introduced ranges. *Biological Invasions* **10**, 1289–1298.

**Meyer, C. P. and Paulay, G.** (2005). DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology* **3**, e422.

**Mitchell, A.** (2011). DNA barcoding is useful for taxonomy: a reply to Ebach. *Zootaxa* **2772**, 67 – 68.

**Mittermeier, R. A., Ganzhorn, J. U., Konstant, W. R., Glander, K., Tattersall, I., Groves, C. P., … Rasoloarison, R. M.** (2008). Lemur diversity in Madagascar. *International Journal of Primatology* **29**, 1607–1656.

**Morand, S.** (2015). (macro-) Evolutionary ecology of parasite diversity: From determinants of parasite species richness to host diversification. *International*

*Journal for Parasitology: Parasites and Wildlife* **4**, 80–87.
**Morand, S. and Guégan, J.-F.** (2000). Distribution and abundance of parasite nematodes: ecological specialisation, phylogenetic constraint or simply epidemiology? *Oikos* **88**, 563–573.
**Morand, S. and Harvey, P. H.** (2000). Mammalian metabolism, longevity and parasite species richness. *Proceedings of the Royal Society B* **267**, 1999–2003.
**Morand, S. and Poulin, R.** (1998). Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology* **12**, 717–727.
**Mouquet, N., Munguia, P., Kneitel, J. M. and Miller, T. E.** (2003). Community assembly time and the relationship between local and regional species richness. *Oikos* **103**, 618–626.
**Muñoz, G., Cribb, T. H. and Mun, G.** (2005). Infracommunity structure of parasites of *Hemigymnus melapterus* (Pisces: Labridae) from Lizard Island, Australia: the importance of habitat and parasite body size. *Journal of Parasitology* **91**, 38–44.
**Mykytowycz, R.** (1962). Epidemiology of coccidiosis (Eimeria spp.) in an experimental population in the Australian wild rabbit, *Oryctolagus cuniculus* (L.). *Parasitology* **52**, 375–395.
**Nalubamba, K. S., Mudenda, N. B. and Malamo, M. R.** (2012). A seasonal survey of gastrointestinal parasites in captive wild impala antelope on a game facility south of Lusaka, Zambia. *Journal of Helminthology* **86**, 418–25.
**Nee, S., Gregory, R. D. and May, R. M.** (1991). Core and satellite specie : theory and artefavts. *Oikos* **62**, 83–87.
**Nichols, E. and Gómez, A.** (2011). Conservation education needs more parasites. *Biological Conservation* **144**, 937–941.
**Nishigori, M.** (1928). The factors which influence the external development of Strongyloides stercoralis and on auto-infection with this parasite. *Journal of Formosan Medical Association* **247**, 1–56.
**Norton, J., Rollinson, D. and Lewis, J. W.** (2004). Patterns of infracommunity species richness in eels, *Anguilla anguilla*. *Journal of Helminthology* **78**, 141–146.
**Nunn, C. L. and Dokey, A. T.-W.** (2006). Ranging patterns and parasitism in primates. *Biology Letters* **2**, 351–4.
**Nunn, C. L., Altizer, S., Jones, K. E. and Sechrest, W.** (2003). Comparative tests of parasite species richness in primates. *The American Naturalist* **162**, 597–614.
**Ogedengbe, J. D., Hanner, R. H. and Barta, J. R.** (2011). DNA barcoding identifies Eimeria species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). *International Journal for Parasitology* **41**, 843–50.
**Osborne, L. C., Monticelli, L. A., Nice, T. J., Sutherland, T. E., Siracusa, M. C., Hepworth, M. R., … Artis, D.** (2014). Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. *Science* **345**, 578–582.
**Pacheco, M. A., Battistuzzi, F. U., Junge, R. E., Cornejo, O. E., Williams, C. V, Landau, I., … Escalante, A. A. (2011).** Timing the origin of human malarias: the lemur puzzle. *BMC Evolutionary Biology* **11**, 299.
**Paterson, S. and Viney, M. E.** (2003). Functional consequences of genetic diversity in Strongyloides ratti infections. *Proceedings of the Royal Society B: Biological Sciences* **270**, 1023–32.
**Patterson, J. E. H. and Ruckstuhl, K. E.** (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology* **140**, 803–13.
**Pedersen, A. B. and Davies, T. J.** (2009). Cross-species pathogen transmission and disease emergence in primates. *EcoHealth* **6**, 496–508.
**Pedersen, A. B. and Fenton, A.** (2007). Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution* **22**, 133–9.
**Pedersen, A. B., Altizer, S., Poss, M., Cunningham, A. a and Nunn, C. L.** (2005). Patterns of host specificity and transmission among parasites of wild primates. *International journal for parasitology* **35**, 647–57.
**Perkins, S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. and Hudson, P. J.** (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology* **33**, 909–917.
**Perkins, S. L., Martinsen, E. S. and Falk, B. G.** (2011). Do molecules matter more than morphology? Promises and pitfalls in parasites. *Parasitology* **138**, 1664–74.
**Pietrock, M. and Marcogliese, D. J.** (2003). Free-living endohelminth stages: at the mercy of environmental conditions. *Trends in Parasitology* **19**, 293–299.
**Pilosof, S., Morand, S., Krasnov, B. R. and Nunn, C. L.** (2014). Potential parasite transmission in multi-host networks based on parasite sharing. *PeerJ PrePrints*.
**Plieskatt, J. L., Deenonpoe, R., Mulvenna, J. P., Krause, L., Sripa, B., … Brindley, P. J.** (2013). Infection with the carcinogenic liver fluke Opisthorchis viverrini modifies intestinal and biliary microbiome. *FASEB journal* **27**, 4572–84.
**Porazinska, D. L., Giblin-Davis, R. M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., …Thomas, W. K.** (2009). Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. *Molecular Ecology Resources* **9**, 1439–1450.

**Porazinska, D. L., Sung, W., Giblin-Davis, R. M. and Thomas, W. K.** (2010a). Reproducibility of read numbers in high-throughput sequencing analysis of nematode community composition and structure. *Molecular Ecology Resources* **10**, 666–76.

**Porazinska, D. L., Giblin-Davis, R. M., Esquivel, A., Powers, T. O., Sung, W. and Thomas, W. K.** (2010b). Ecometagenetics confirm high tropical rainforest nematode diversity. *Molecular Ecology* **19**, 5521–30.

**Porazinska, D. L., Giblin-Davis, R. M., Powers, T. O. and Thomas, W. K.** (2012). Nematode spatial and ecological patterns from tropical and temperate rainforests. *PloS One* **7**, e44641.

**Poulin, R.** (1997). Population abundance and sex ratio in dioecious helminth parasites. *Oecologia* **111**, 375–380.

**Poulin, R.** (2001). Interactions between species and the structure of helminth communities. *Parasitology* **122**, 3–11.

**Poulin, R.** (2005). Detection of interspecific competition in parasite communities. *Journal of Parasitology* **91**, 1232–1235.

**Poulin, R.** (2007). *Evolutionary ecology of parasites*. Princeton University Press, Princeton, NJ, USA.

**Poulin, R.** (2010). Decay of similarity with host phylogenetic distance in parasite faunas. *Parasitology* **137**, 733–41.

**Poulin, R.** (2014). Parasite biodiversity revisited: frontiers and constraints. *International Journal for Parasitology* **44**, 581–589.

**Poulin, R. and Keeney, D. B.** (2008). Host specificity under molecular and experimental scrutiny. *Trends in Parasitology* **24**, 24–8.

**Poulin, R. and Morand, S.** (2000). The diversity of parasites. *The Quarterly Review of Biology* **75**, 277–293.

**Poulin, R. and Morand, S.** (2004). *Parasite biodiversity*. Smithsonian Institution Press, Washington DC, USA.

**Prenter, J., Macneil, C., Dick, J. T. a and Dunn, A. M.** (2004). Roles of parasites in animal invasions. *Trends in Ecology & Evolution* **19**, 385–90.

**Prichard, R. and Tait, A.** (2001). The role of molecular biology in veterinary parasitology. *Veterinary Parasitology* **98**, 169–94.

**Prosser, S. W. J., Velarde-Aguilar, M. G., León-Règagnon, V. and Hebert, P. D. N.** (2013). Advancing nematode barcoding: a primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. *Molecular Ecology Resources* **13**, 1108–15.

**Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glöckner, F. O.** (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**, D590–6.

**Quentin, J.-C. and Durette-Desset, M.-C.** (1974). Helminthofaune des Rongeurs Cricetides malgaches du genre *Brachyuromys*. *Bulletin du Muséum National d'Histoire Naturelle, Paris Serie Zoologie* **135,** 89-111.

**Quince, C., Lanzen, A., Davenport, R. J. and Turnbaugh, P. J.** (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* **12**, 38.

**Radespiel, U., Schaber, K., Kessler, S. E., Schaarschmidt, F. and Strube, C.** (2015). Variations in the excretion patterns of helminth eggs in two sympatric mouse lemur species (Microcebus murinus and M. ravelobensis) in northwestern Madagascar. *Parasitology Research* **114**, 941–54.

**Raharivolona, B.M., Rakotondravao and Ganzhor**n, J.U. (2007) Gastrointestinal parasites of small mammals in the littoral forest of Mandena. In: *Biodiversity, Ecology and Conservation of Littoral Forest Ecosystems in Southeastern Madagascar, Tolagnaro (Fort Dauphin)*. (eds Ganzhorn, J.U., Goodman, S.M. and Vincelette, M.), pp. 247-258. Smithsonian Institution, Washington D.C., USA.

**Raharivololona, B. M. and Ganzhorn, J. U.** (2009). Gastrointestinal parasite infection of the gray mouse lemur (Microcebus murinus) in the littoral forest of Mandena, Madagascar: effects of forest fragmentation and degradation. *Madagascar Conservation & Development* **4**, 103–112.

**Raharivololona, B. and Ganzhorn, J.** (2010). Seasonal variations in gastrointestinal parasites excreted by the gray mouse lemur Microcebus murinus in Madagascar. *Endangered Species Research* **11**, 113–122.

**Rasambainarivo, F. T., Gillespie, T. R., Wright, P. C., Arsenault, J., Villeneuve, A., & Lair, S**. (2013). Survey of Giardia and Cryptosporidium in lemurs from the Ranomafana National Park, Madagascar. *Journal of Wildlife Diseases* **49**, 741–3.

**Read, C. P.** (1951). "Crowding effect" in tapeworm infections. *Journal of Parasitology* **37**, 174–178.

**Reuter, O. M.** (1913). *Lebensgewohnheiten und Instinkte der Insekten bis zum Erwachen der sozialen Instinkte*. Friendlander & Sohn, Berlin, Germany.

**Rigaud, T., Perrot-Minnot, M.-J. and Brown, M. J. F.** (2010). Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proceedings of the Royal Society B: Biological Sciences* **277**, 3693–3702.

**Roberts, L. S.** (2000). The crowding effect revisited. *Journal of Parasitology* **86**, 209–211.

**Roberts, M. L., Buchanan, K. L. and Evans, M. R.** (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* **68**, 227–239.

**Rózsa, L., Reiczigel, J. and Majoros, G.** (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228–232.

**Ryan, J. M., Creighton, G. K. and Emmons, L. H.** (1993). Activity patterns of two species of Nesomys (Muridae: Nesomyinae ) in a Madagascar rain forest. *Journal of Tropical Ecology* **9**, 101–107.

**Rynkiewicz, E. C., Pedersen, A. B. and Fenton, A.** (2015). An ecosystem approach to understanding and managing within-host parasite community dynamics. *Trends in Parasitology*.

**Råberg, L.** (2014). How to live with the enemy: Understanding tolerance to parasites. *PLoS Biology* **12**, e10011989.

**Råberg, L., Sim, D. and Read, A. F.** (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* **318**, 812–814.

**Samonds, K. E., Godfrey, L. R., Ali, J. R., Goodman, S. M., Vences, M., Sutherland, M. R., … Krause, D. W.** (2012). Spatial and temporal arrival patterns of Madagascar's vertebrate fauna explained by distance, ocean currents, and ancestor type. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 5352–7.

**Sandground, B. J. H.** (1926). Biological studies on the life-cycle in the genus Strongyloides grassi, 1879. *American Journal of Epidemiology* **6**, 337–388.

**Sandosham, A.A.** (1950). On *Enterobius* and some related species from primates and rodents. *Journal of Helminthology* **24**, 171-204.

**Santos, A. M. C., Besnard, G. and Quicke, D. L. J.** (2011). Applying DNA barcoding for the study of geographical variation in host-parasitoid interactions. *Molecular Ecology Resources* **11**, 46–59.

**Schad, J., Ganzhorn, J. U. and Sommer, S**. (2005). Parasite burden and constitution of major histocompatibility complex in the Malagasy mouse lemur, *Microcebus murinus*. *Evolution* **59**, 439-450.

**Schalk, G. and Forbes, M. R.** (1997). Male biases in parasitism of mammals: effects of study type, host age , and parasite taxon. *Oikos* **78**, 67–74.

**Schmid, J.** (1998). Fluctuating sexual dimorphism and differential hibernation by sex in a primate , the gray mouse lemur (Microcebus murinus). *Behavioral Ecology and Sociobiology* **43**, 125–132.

**Schmid-Hempel, P.** (2003). Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences* **270**, 357–66.

**Schmid-Hempel, P.** (2011). *Evolutionary parasitology: the integrated study of infections, immunology, ecology and genetics*. Oxford University Press, Oxford, UK.

**Schulte, R. D., Makus, C., Hasert, B., Michiels, N. K., & Schulenburg, H.** (2011). Host-parasite local adaptation after experimental coevolution of Caenorhabditis elegans and its microparasite Bacillus thuringiensis. *Proceedings of the Royal Society B: Biological Sciences* **278**, 2832–9.

**Schülke, O. and Ostner, J.** (2006). Physiological ecology of cheirogaleid primates: variation in hibernation and torpor. *Acta Ethologica* **10**, 13–21.

**Schwensow, N., Dausmann, K., Eberle, M., Fietz, J. and Sommer, S.** (2010). Functional associations of similar MHC alleles and shared parasite species in two sympatric lemurs. *Infection, Genetics and Evolution* **10**, 662-8.

**Shaw, D. J., Grenfell, B. T. and Dobson, A.P.** (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* **117**, 597–610.

**Shaw, D. J., and Dobson, A. P.** (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111,** S111–3.

**Sheldon, B. C. and Verhulst, S.** (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**, 317–321.

**Shrestha, S., King, A. A. and Rohani, P.** (2011). Statistical inference for multi-pathogen systems. *PLoS Computational Biology* **7**, e1002135.

**Silva, N. R. R. Da, Silva, M. C. Da, Genevois, V. F., Esteves, A. M., Ley, P. De, Decraemer, W., Rieger, T. T. and Correia, M. T. D. S.** (2010). Marine nematode taxonomy in the age of DNA: the present and future of molecular tools to assess their biodiversity. *Nematology* **12**, 661–672.

**Simões, R. O., Souza, J. G. R., Maldonado, A. and Luque, J. L.** (2010). Variation in the helminth community structure of three sympatric sigmodontine rodents from the coastal Atlantic Forest of Rio de Janeiro, Brazil. *Journal of Helminthology* 1–8.

**Soler, J. J., de Neve, L., Pérez-Contreras, T., Soler, M. and Sorci, G.** (2003). Trade-off between immunocompetence and growth in magpies: an experimental study. *Proceedings of the Royal Society B: Biological Sciences* **270**, 241–8.

**Stear, M. J., Bishop, S. C., Duncan, J. L., Mckellar, Q. A. and Murray, M.** (1995). The repeatability of faecal egg counts, peripheral eosinophil counts, and plasma pepsinogen concentrations during deliberate infection with Ostertagia circumcincta. *International Journal for Parasitology* **25**, 375–380.

**Stear, M. J., Abuagob, O., Benothman, M., Bishop, S. C., Innocent, G., Kerr, A. and Mitchell, S.** (2006). Variation among faecal egg counts following

natural nematode infection in Scottish Blackface lambs. *Parasitology* **132**, 275–280.

**Stephenson, L. S., Latham, M. C. and Ottesen, E. A.** (2000). Malnutrition and parasitic helminth infections. *Parasitology* **121**, S23–38.

**Strachan, D. P.** (1989). Hay fever, hygiene, and household size. *British Medical Journal* **299**, 1259–60.

**Symondson, W. O. C.** (2002). Molecular identification of prey in predator diets. *Molecular Ecology* **11**, 627–41.

**Szalai, A. J., & Dick, T. A.** (1989). Differences in numbers and inequalities in mass and fecundity during the egg-producing period for Raphidascaris acus (Nematoda: Anisakidae). *Parasitology* **98**, 489–495.

**Tanaka, R., Hino, A., Tsai, I.J., Palomares-Rius, J.E., Yoshida, A., Ogura, Y., … Kikuchi, T.** (2014) Assessment of helminth biodiversity in wild rats using 18S rDNA based metagenomics. *PLoS ONE* **9**, e110769.

**Telfer, S., Bown, K. J., Sekules, R., Begon, M., Hayden, T. and Birtles, R.** (2005). Disruption of a host-parasite system following the introduction of an exotic host species. *Parasitology* **130**, 661–668.

**Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S. and Begon, M.** (2010). Species interactions in a parasite community drive infection risk in a wildlife population. *Science* **330**, 243–246.

**Thompson, R. C. a, Lymbery, a J. and Smith, A.** (2010). Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology* **40**, 1163–70.

**Tollenaere, C., Brouat, C., Duplantier, J.-M., Rahalison, L., Rahelinirina, S., Pascal, M., … Cosson, J.-F.** (2010). Phylogeography of the introduced species Rattus rattus in the western Indian Ocean, with special emphasis on the colonization history of Madagascar. *Journal of Biogeography* **37**, 398–410.

**Torchin, M. E., Lafferty, K. D., Dobson, A. P., Mckenzie, V. J. and Kuris, A. M.** (2003). Introduced species and their missing parasites. *Nature* **421**, 628–630.

**Torgerson, P. R. and Macpherson, C. N. L.** (2011). The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary Parasitology* **182**, 79–95.

**Turner, A. K., Beldomenico, P. M., Bown, K., Burthe, S. J., Jackson, J. A., Lambin, X. and Begon, M.** (2014). Host-parasite biology in the real world: the field voles of Kielder. *Parasitology* **141**, 997–1017.

**Ulrich, W. and Gotelli, N. J.** (2013). Pattern detection in null model analysis. *Oikos* **122**, 2–18.

**Valentini, A., Pompanon, F. and Taberlet, P.** (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution* **24**, 110–7.

**Walk, S. T., Blum, A. M., Ewing, S. A.-S., Weinstock, J. V and Young, V. B.** (2010). Alteration of the murine gut microbiota during infection with the parasitic helminth Heligmosomoides polygyrus. *Inflammatory Bowel Diseases* **16**, 1841–9.

**Vaumourin, E., Vourc'h, G., Telfer, S., Lambin, X., Salih, D., Seitzer, U., … Gasqui, P.** (2014). To be or not to be associated: power study of four statistical modeling approaches to identify parasite associations in cross-sectional studies. *Frontiers in Cellular and Infection Microbiology* **4**, 1–11.

**Vences, M.** (2004). Origin of Madagascar's extant fauna: A perspective from amphibians, reptiles and other non-flying vertebrates. *Italian Journal of Zoology* **71**, 217–228.

**Vences, M., Vieites, D. R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M. and Meyer, A.** (2003). Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society B: Biological Sciences* **270**, 2435–42.

**Vences, M., Kosuch, J., Rödel, M.-O., Lötters, S., Channing, A., Glaw, F. and Böhme, W.** (2004). Phylogeography of Ptychadena mascareniensis suggests transoceanic dispersal in a widespread African- Malagasy frog lineage. *Journal of Biogeography* **31**, 593–601.

**Wikelski, M., Foufopoulos, J., Vargas, H. and Snell, H.** (2004). Galápagos birds and diseases: invasive pathogens as threats for island species. *Ecology and Society* **9**, 5.

**Wilson, K., Grenfell, B. T., Pilkington, J. G., Boyd, H. E. and Gulland, F. M.** (2004). Parasites and their impact. In *Soay sheep: dynamics and selection in an island population* (ed. Clutton-Brock, T. H. and Pemberton, J. M.), pp. 113–165. Cambridge University Press, Cambridge, UK.

**Wilson, J. J., Rougerie, R., Schonfeld, J., Janzen, D. H., Hallwachs, W., Hajibabaei, M., … Hebert, P. D. N.** (2011). When species matches are unavailable are DNA barcodes correctly assigned to higher taxa? An assessment using sphingid moths. *BMC Ecology* **11**, 18.

**Wimmer, B., Craig, B. H., Pilkington, J. G. and Pemberton, J. M.** (2004). Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. *International Journal for Parasitology* **34**, 625–631.

**Windsor, D. A.** (1998). Most of the species on Earth are parasites. *International Journal for Parasitology* **28**, 828–830.

**Viney, M. E. and Graham, A. L.** (2013). Patterns and processes in parasite co-infection. *Advances in*

*Parasitology* **82**, 321–369.

**Vizoso, D. B., and Ebert, D.** (2005). Phenotypic plasticity of host-parasite interactions in response to the route of infection. *Journal of Evolutionary Biology* **18**, 911–21.

**Vogler, A. J., Chan, F., Wagner, D. M., Roumagnac, P., Lee, J., Nera, R., … Keim, P.** (2011). Phylogeography and molecular epidemiology of Yersinia pestis in Madagascar. *PLoS Neglected Tropical Diseases* **5**, e1319.

**Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. and Lal, A. A.** (1998). Wild primate populations in emerging infectious disease research: the missing link? *Emerging Infectious Diseases* **4**, 149–58.

**Wood, E. L. D., Matthews, J. B., Stephenson, S., Slote, M. and Nussey, D. H.** (2013). Variation in fecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation. *Parasitology* **140**, 115–128.

**Woolhouse, M. E. J., Dye, C., Etard, J.-F., Smith, T., Charlwood, J. D., Garnett, G. P., … M, A. R.** (1997). Heterogeneities in the transmission of infectious agents: implications ofr the design of control programs. *Proceedings of National Academies of Sciences* **94**, 338–342.

**Wright, P. C. and Andriamihaja, B.** (2002). Making a rain forest national park work in Madagascar: Ranomfana National Park and its long-term research commitment. In *Making parks work: strategies for preserving tropical nature* (ed. Terborgh, J., van Schaik, C., Davenport, L., and Rao, M.), pp. 112–136. Island Press, Washington DC, USA.

**Wright, P. C., Erhart, E. M., Tecot, S., Baden, A. L., Arrigo-Nelson, S. J., Herrera, J., … Zohdy, S.** (2012). *Long-term field studies of primates*. (ed. Kappeler, P. M. and Watts, D. P.) Springer Berlin Heidelberg, Berlin, Heidelberg

**Wright, P. C., Andriamihaja, B., King, S. J., Guerriero, J. and Hubbard, J.** (2014). Lemurs and tourism in Ranomafana National Park, Madagascar: economic boom and other consequences. In *Primate tourism: a tool for conservation?* (ed. Russon, A. and Wallis, J.), pp. 123–146. Cambridge University Press, Cambridge, UK.

**Yanagida, T., Carod, J.-F., Sako, Y., Nakao, M., Hoberg, E. P., and Ito, A.** (2014). Genetics of the pig tapeworm in madagascar reveal a history of human dispersal and colonization. *PloS one* **9**, e109002.

**Zohdy, S., Kemp, A. D., Durden, L. a, Wright, P. C. and Jernvall, J.** (2012). Mapping the social network: tracking lice in a wild primate (Microcebus rufus) population to infer social contacts and vector potential. *BMC Ecology* **12**, 4.

**Zohdy, S., Gerber, B. D., Tecot, S., Blanco, M. B., Winchester, J. M., Wright, P. C. and Jernvall, J.** (2014). Teeth, sex, and testosterone: aging in the world's smallest primate. *PLoS One* **9**, e109528.

**Zuk, M.** (2009). The sicker sex. *PLoS Pathogens* **5**, e1000267.