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Article

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ARTICLE

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PLATINUM OPEN ACCESS

Effect of socio-ecological factors and parasite infection on body condition of Brown Mouse Lemur *Microcebus rufus* (Mammalia: Primates: Cheirogaleidae)

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Abstract: Various studies in ecology have shown the relationship between body condition and parasitic loads in nonhuman primates, however, little information is available regarding prosimians such as lemurs. In this study, the synergistic effect of parasite infection and socio-ecological factors on the body condition of *Microcebus rufus* in the family Cheirogaleidae was analyzed in Ranomafana National Park in southeastern Madagascar. This lemur species is characterized by its ability to adapt to different types of forest, and by seasonal fattening. Based on the factors considered, this species is, therefore, a good model for the study of body condition and ecology of infectious diseases in lemurs. Floatation and direct observation techniques were used for examination of parasite infection. Two indices considering body condition were analyzed: volume index (VI) and condition index (CI), the residual between the mass observed and the corrected mass. The generalized linear mixed model (GLMM) was used to model the synergistic effect of parasite infections and socio-ecological factors on variation in body condition, with the identity of individuals used as a random factor. We identified five species of helminths, one species of protist, and one species of lice which infected the 204 mouse lemurs captured. There was a sexual difference for all measures of the parasite infection. The more parasite species an individual was infected with, the smaller was its body size according to the Volume Index that reflects deposits of subcutaneous fat. Individuals with more positive Condition Index values, particularly females, excreted more parasite eggs or oocyst in their faecal matter. The results suggest that an individual's body condition constitutes an indicator of risk of parasite infection and transmission.

Keywords: Condition index, infectious disease, Ranomafana National Park, southeastern Madagascar, volume index.

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For **Author details** and **Author contribution**, see end of this article.

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INTRODUCTION

The knowledge of body condition of an animal is of considerable importance in ecological studies and in the detection of diseases (Coop & Holmes 1996; Alzaga et al. 2008; Munyeme et al. 2010), as a tool for wildlife management (Ezenwa et al. 2010), and as an important indicator for numerous infectious diseases that affect the fitness of an individual (Sheldon & Verhulst 1996). Animals in a poor condition are often loaded with more parasites than individuals in a better condition (Wilford et al. 1986; Chapman et al. 2006; Tompkins et al. 2011). In addition, they are particularly susceptible to parasitic infections, leading to a "vicious cycle" of continuous parasitic infections and deteriorating health (Beldomenico & Begon 2010).

Thus, parasites play a key role in tropical ecosystems and affect not only the ecology and the evolution of intra- and interspecific interactions (Kappeler & Van Schaik 2002; Kappeler et al. 2015), but also the regulations of the host species health (Esch & Fernandez 1993; Hudson et al. 1998; Hochachka & Dhondt 2000; Hudson et al. 2002; Loudon et al. 2006). Understanding the functioning of parasite populations and the condition of the hosts within ecosystems is crucial, both in terms of parasite and host behavior (Schwitzer et al. 2010) and with regard to the epidemiological risk to host health (Silk 1986; Sanchez-Villagra et al. 1998; Gillespie & Chapman 2008).

Although studies on body condition, feeding, parasites, and disease have been conducted on monkeys in captivity and in the wild (Chapman et al. 2006; Altizer et al. 2007), little information has been collected about body condition indices, risk factors for parasite infection, and the relationship between body condition and parasites in prosimian primates such as lemurs, particularly in nocturnal species (Rafalinirina et al. 2015). We chose to study a member of the family Cheirogaleidae (Brown Mouse Lemur *Microcebus rufus* Vulnerable) in Ranomafana National Park (RNP) in southeastern Madagascar (Image 1). This species is widespread and easily adaptable to secondary forests and degraded vegetation, thus it is possible for it to encounter different types of parasites. Additionally, it is characterized by seasonal fattening (though not for all individuals at this site) in preparation for the dry season followed by torpor (Atsalis 1999). Therefore, it is a good model to show the relation between body condition and parasite infection ecology of lemurs, according to socio-ecological factors (sex, year of study, site, and period of capture) present in a natural environment for conservation management. Indeed, the purpose of this study was to examine indices of body condition, parasitic infection, and the synergistic effect of socio-ecological factors and parasitic infection on body condition. The analysis of morpho-physiological characters and the examination of feces seem to be the most effective methods for achieving this objective.

Image 1. Brown Mouse Lemur *Microcebus rufus*

MATERIALS AND METHODS

Study Site

Ranomafana National Park (RNP) is located in southeastern Madagascar. It is 65km northeast of the city of Fianarantsoa and adjacent to the village of Ranomafana, midway along the National Road 25 connecting Fianarantsoa and Mananjary. It lies in the geographical position Latitude 47.333^{0}E and Longitude 21.266^{0}S with an altitude between 400m and 1417m (Wright 1992; Wright & Andriamihaja 2002). The Park covers an area of 41,613ha, within which we collected samples at three sites: the Ranofady circuit (47.420^{0}E, 21.260^{0}S); the campsite of the Valbio Center (47.419^{0}E, 21.253^{0}S); and the Talatakely tourist site (47.421^{0}E, 21.262^{0}S) (Wright et al. 2009) (Fig. 1).

Data collection

Microcebus rufus were captured five days per week using Sherman traps (XLR, Sherman Trap Inc., Florida, USA 22.2 x 6.6 x 6.6 cm) from August to December in 2012, 2013 and 2015:

Data were collected according to three selected periods: Period 1 (before mating: mid-August to the beginning of October), Period 2 (mating, defined by the dates between which first and last vaginal opening were observed, most of October), and Period 3 (after mating: from November to December).

We measured the following morphometric points

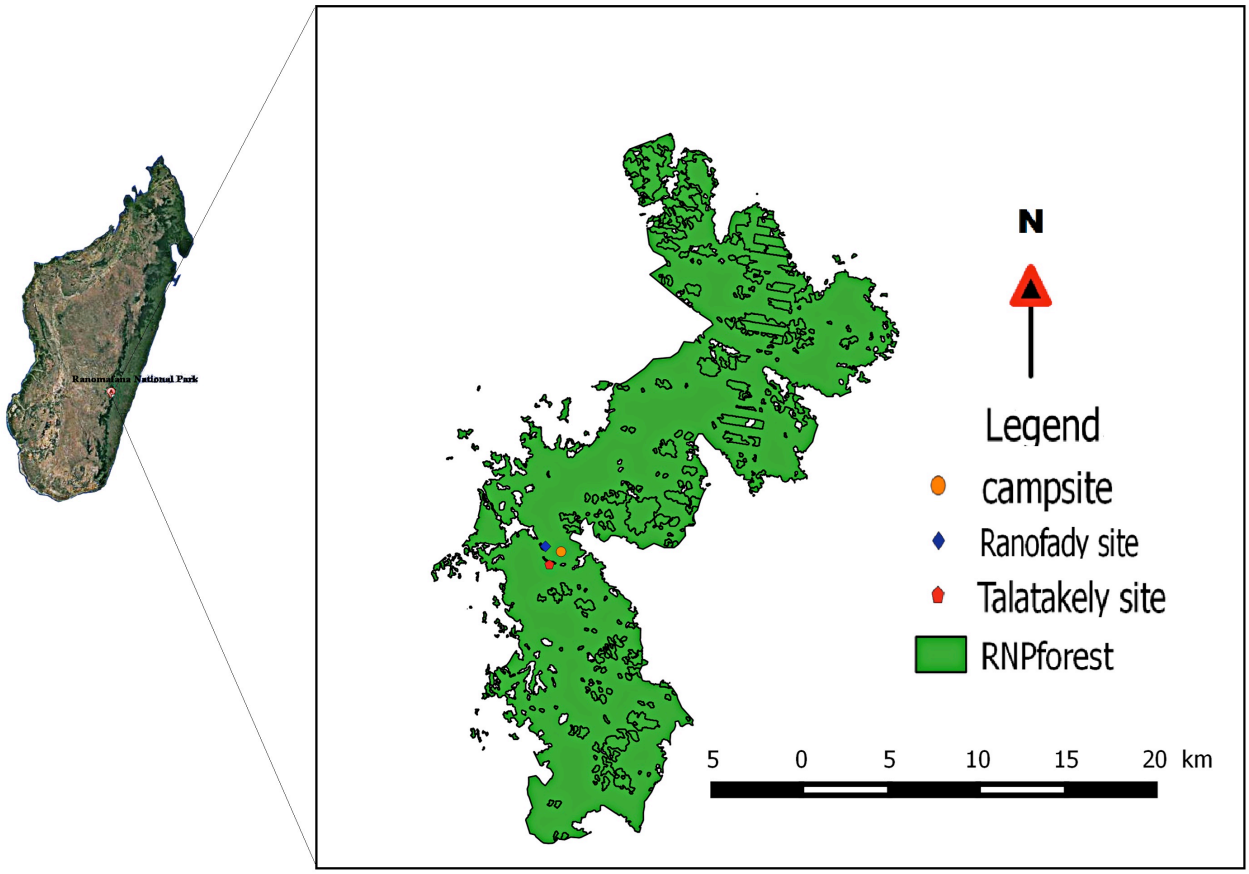

Figure 1. Study area in Ranomafana National Park, southeastern Madagascar.

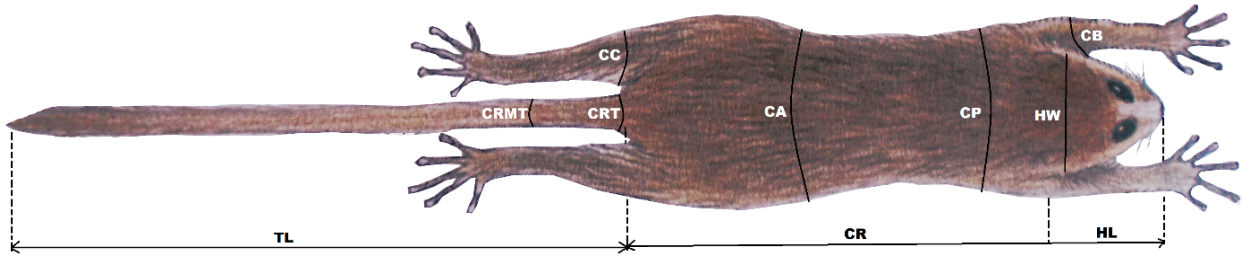

Figure 2. Different morphometric points for *Microcebus rufus*.

at the circumference of: the base of the tail (CRT), the midpoint of the tail (CRMT), the chest (CP), the hip (CA), the biceps (CB), and the thigh (CC). We also, measured body mass (M), body length (BL, from the prominent occipital point to the base of the tail), the length of the tail (TL, from the base to the tail tip), the length of the head (HL, from the tip of the nose to the prominent occipital point), and the width of the head (HW, between the two temples) (Fig. 2).

We also calculated the approximate body volume by the formula established by (Labocha et al. 2014):

$BV = 1 / 3\pi TBL(RC^2 + RC^* RH + RH^2)$

BV: Body volume, TBL: Total Body Length (BL+TL+HL), RC: Ray of Chest, RH: Ray of Hip.

Individual fecal samples were collected inside the traps, in bags, or directly from the anus for analysis. Because with the small lemurs only feces less than 1g were recovered: ~0.3g of fecal matter was recovered from each individual (Kessler et al. 2015; Radespiel et al. 2015).

Analysis

(a) Fecal analysis

We analyzed fecal material without preservatives in the ValBio Center lab, following Gillespie's method

(2006). The modified floatation technique using Sheather's solution (454g table sugar, 355ml tap water, 6ml formaldehyde) (Dryden et al. 2005) allows for the counting of eggs, larvae, and parasite oocysts in McMaster slides (Weber UK International Scientific).

Three parameters were used to determine parasite infestation: parasite species richness (PSR) (number of species or types of parasites encountered in a host individual. The index reflects the polyparasitism of individual hosts); parasite prevalence (number of host individuals infested by a particular parasite divided by the number of hosts examined multiplied by 100); parasite abundance (number of parasite eggs or parasitic elements per gram of feces).

For confirmation, we used the key determination of nematodes eggs, tapeworms and protozoan cysts published by Raharivololona (2009), as well as the genetic analysis methodology done by Aivelo (2015).

(b) Research on Ectoparasites

Microcebus rufus from Ranomafana exhibit ectoparasites such as lice (*Lemurpediculus verruculosus*) (Durden et al. 2010). In this study, the presence of this ectoparasite was verified for each individual, most often encountered at the abdomen, genitals, ear, and eyebrow. Ectoparasites were counted and quantified according to abundance on the body (Rafalinirina et al. 2015).

(c) Body Condition Estimation

For *Microcebus rufus*, we determined body condition based on the non-destructive estimation method (Green 2001; Stevenson & Woods 2006). We used body mass, the size, or the appearance of energy reserves. According to the recommendation of Peig & Green (2009), the body condition of an individual is estimated from the scaled mass index (SMi):

$$SMi = Mi\left[\frac{TBLo}{TBLi}\right]^{bSMA}$$

with $bSMA = b/r$ where Mi and TBLi are the body mass and total body length of individual i respectively; bSMA is the scaling exponent estimated by the standardized major axis regression of M on TBL, and TBLo is the arithmetic mean of TBL for our study population.

The difference or residual (CI) between the observed body mass M and the mass corrected for the individual size SMi gives the loss or mass gain in this research. Therefore, the value of the negative CI reflects a loss of mass reflecting poor animal health, while its positive value indicates a gain of mass reflecting good health.

Microcebus is one of the only primates that stores fats in the caudal section, used as a source of energy. We calculated the Volume index (VI) resulting from the principal component analysis of circumference measurements (CRT, CRMT, CB, CC), including body volume (BV) (Appendix 1).

(d) Statistical Analysis

The capture-mark-recapture technique was utilized, and thus each individual of *Microcebus rufus* contributes a maximum of data points in each period for all the data. Therefore, if there are multiple data points available for an individual during a period, then the average of the parameters studied will be used. For data recording and manipulation, we used Excel, after which the arranged data was transferred to SPSS version 22.0 (SPSS Inc., an IBM Company product, Chicago Illinois) for description, analysis and statistical modeling.

A principal component analysis was used to condense information from collinear variables (Zuur et al. 2010). In addition, GLMM was used to understand and analyze the effect of parasite infection measures and socio-ecological factors on the change in body condition index, with normal distribution and identity link function. In the analysis, the identity of the individuals (ID) was used as a random effect. All statistical analyses were two tailed and $P< 0.05$ was considered statistically significant.

RESULTS

The parasites of *Microcebus rufus*

In the 204 lemur individuals captured, five helminths and one protozoan species of gastrointestinal parasites were identified. The helminthofauna includes four nematodes, three Strongylidae species (*Strongyloides* sp., *Trichuris* sp., *Trichostrongylus* sp.), a species of the order Ascaridida (*Ascaris* sp.), and one cestode belonging to the genus Hymenolepis (Image 2). The protozoan species identified was coccidia.

In addition to the aforementioned gastrointestinal parasites, one species of lice (ectoparasites) of the order Phthiraptera, *Lemurpediculus verruculosus,* was found (Image 3).

(a) Parasite prevalence of *Microcebus rufus*: Among the parasite species inventoried, the prevalence of *Strongyloides* sp., *Hymenolepis* sp., *Lemurpediculus verruculosus,* and Coccidia were the most dominant (Fig. 3), which is to say that *Microcebus rufus* in Ranomafana National Park are most infested by these three gastrointestinal parasite species and this species of lice.

For the overall prevalence of these parasites in the

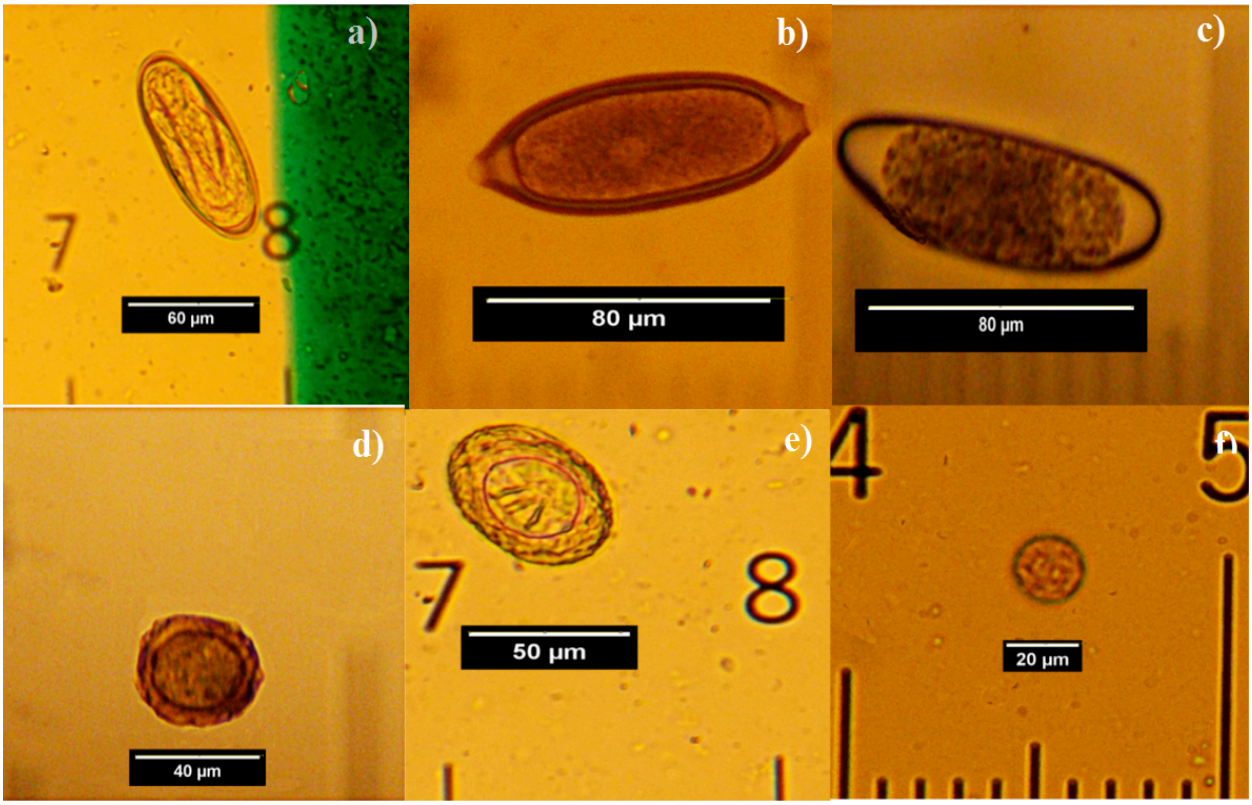

Image 2. Gastrointestinal parasite found in feces of *Microcebus rufus*: (a) *Strongyloides* sp. | (b) *Trichuris* sp. | (c) *Trichostrongylus* sp. | (d) *Ascaris* sp. | (e) *Hymenolepis* sp. | (f) a non-identified oocyst of Coccidia.

Image 3. An ectoparasite *Lemurpediculus verruculosus* found in *Microcebus rufus.*

Park, there is a significant infestation of *Strongyloides* sp.: 43.69% of the captured individuals were infested (173 individuals out of 204) by this parasite, 16.67% (66 out of 204 individuals) were infested by the *Hymenolepis* sp., and 23.99% (95 out of 204 individuals) and 11.87% (47 out of 204) were infested with ectoparasite and Coccidia.

The nematode is most widespread parasite, followed by ectoparasites, cestode and protozoans in the *Microcebus rufus* of Ranomafana National Park,. Therefore, there is a significant risk of epidemic in the area.

(b) Parasite abundance and species richness of *Microcebus rufus*: Males had the highest parasite abundance number for all parasites (Appendix 2) which presents a risk in the propagation of these parasites. We also observed that the group in Ranofady excreted many *Strongyloides* sp. eggs in their fecal matter, the individuals in Talatakely excreted the most cestode eggs and the lice were there in abundance. Therefore, there are significant incidences of these parasites in these sites. Moreover, the result showed an abundance of parasites during the mating period, which means a high risk of infestation. In addition, there was an increase in the number of nematode eggs in the fecal matter of *Microcebus rufus* during the study years, thus a proliferation of infections. However, a regression of the number of lice that infect the body has been observed. The cestodes were not present in the year 2013 but reappeared in 2015.

For the specific richness of parasites, this index reflects polyparasitism in a host individual. In this study, we observed that 63.23% of *Microcebus rufus* present a polyparasitism – that is to say that 129 of the 204 captured are infected by at least two species of parasites

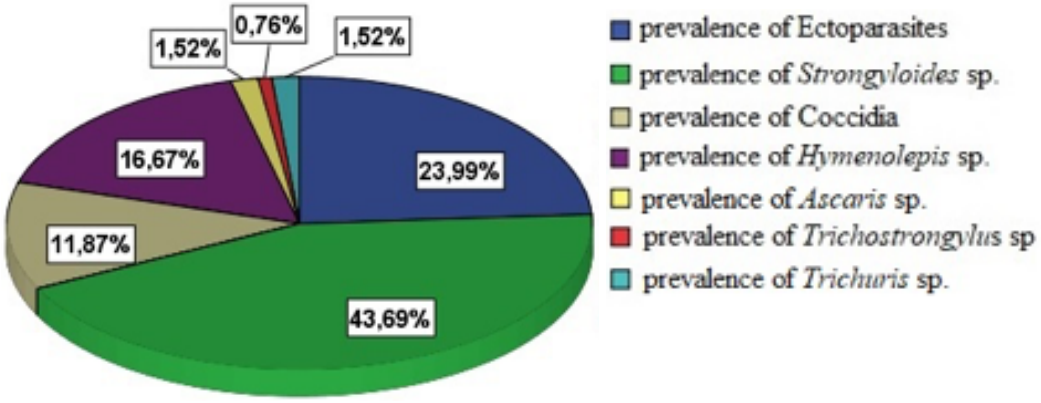

Figure 3. Prevalence of parasite infection in *Microcebus rufus* at RNP.

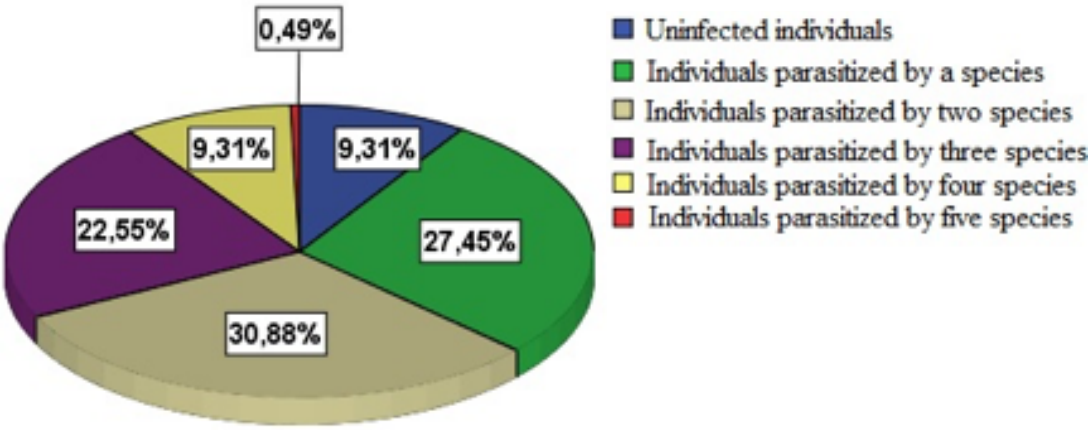

Figure 4. Frequency diagram of the parasites species richness of *Microcebus rufus* at the RNP.

(Fig. 4). Specifically, 63 individuals harbored two distinct parasite species (30.88%), 46 host three (22.5%), and one hosts five species (0.49%).

We found a positive linear correlation between indices of parasitic infections. In order to synthesize all this information on the correlations between parasite infection indices in *Microcebus rufus*, a principal component analysis for quantitative variables (parasite abundance) was carried out (Appendix 3). This allows condensing information on these variables according to synthetic factors (or components), analyzing and determining risk factors in statistical modeling.

Effects of socio-ecological factors and measures of parasitic infections on the body condition of *Microcebus rufus*

(a) Effects of socio-ecological factors and measures of parasitic infections on the variation of the volume index (VI): There was a significant effect of the species richness on the VI (F = 6.53, p = 0.012) (Table 1). The PSR had a negative impact on the body condition index (b = -0.18, t = -2.56, p = 0.012) (Appendix 4). This means that for an infestation of an additional parasite species, the Volume Index for *Microcebus rufus* decreases significantly by 0.18.

The interaction between year of study and sex had a significant impact on the variation of the VI (F = 7.17, p = 0.001), as well as for the combinatorial effect of sex and period (F = 5.81, p = 0.004). With the predictors continuously fixed at the following values: PSR = 2, parasite abundance = 0.02, it is estimated that females during the year 2013 have more than 1.26 of VI compared to that of the males of the same year and the individuals of the year 2015. That is to say that during that year, females were much larger than males. In addition, the female individuals before (b = -1.05, t = -2.95, p = 0.004) and during the mating period (b = -0.92, t = -2.86, p = 0.005) (Appendix 4) had low volume indices (less bulky) compared to those after the mating period and males during all periods.

(b) Effects of socio-ecological factors and measures of parasitic infections on the change in body condition

Table 1. General linear mixed model (GLMM) of variation VI in *Microcebus rufus*.

Dependent variable	Predictors	*F*	df	*p*
Volume Index	Year of study	0.96	2	0.387
	Sex	0.94	1	0.334
	Period	1.68	2	0.190
	Site	1.23	2	0.295
	PSR	6.53	1	**0.012**
	Abundance Parasites Factor	0.00	1	0.971
	Year of study * sex	7.17	2	**0.001**
	Year of study * period	0.11	3	0.954
	Year of study * site	0.01	1	0.915
	Sex * period	5.81	2	**0.004**

Distribution of probability: Normal, identity link function | df - degree of freedom | PSR - Parasites Specific Richness.

Table 2. General linear mixed model (GLMM) of CI variation in *Microcebus rufus*.

Dependent variable	Predictors	*F*	df	*p*
CI	Year of study	7.74	2	**0.001**
	Sex	0.07	1	0.796
	Period	0.79	2	0.457
	Site	5.07	2	**0.007**
	PSR	0.05	1	0.822
	Factor abundance of parasite	4.15	1	**0.043**
	Year of study * sex	17.42	2	**0.000**
	Year of study * period	0.20	3	0.899
	Year of study * site	2.22	1	0.138
	Sex * period	0.43	2	0.650

Distribution of probability: Normal, identity link function | df - degree of freedom | PSR - Parasites Specific Richness.

index (CI): A significant variation in body condition was observed for the year of study (F = 7.74, p = 0.001). The populations of *Microcebus rufus* during the years 2012 and 2013 lost respectively 3.75g (t = -3.30, p = 0.001) and 4.39g (t = -3.40, p = 0.001) of body condition compared to those of 2015. A significant variation was also observed according to site (F = 5.07, p = 0.007), as well as for the combinatorial effect of year of study and sex (F = 17.42, p = 0.000). The group of *Microcebus* in the Ranofady site lost a significant 6.45g (t = -5.44, p = 0.000) of the body condition (CI) compared to those of the Talatakely site. Moreover, it is estimated that female *Microcebus rufus* individuals from 2013 and 2015 are in more optimal condition compared to males of the same year (Appendix 5).

Lastly, we observed significant effect of the parasite infection measure "parasite abundance" on the change in body condition (F = 4.15, p = 0.043) (Table 3). The marginal impact of this parasite infection measure on CI was positive (b = 0.60, t=2.04, p=0.043). This means that if the body condition of the infected *Microcebus* increases by 0.60g, then it will have a secretion of an egg or oocyst number per gram of feces.

DISCUSSION

Parasitic infections are a critical part of the study of conservation biology (May 1988). Parasites, by their very nature, rely on host resources and may affect host survival and reproduction indirectly by reducing the body condition of the host (Coop & Holmes 1996; Neuhaus 2003; Gillespie & Chapman 2005). Individuals in poor condition are unable to resist parasitic infections because of the energy expenditure required for immune defense (Martin et al. 2003). To our knowledge, there are still only a few studies on the effects of gastrointestinal parasites on body condition and socio-ecological factors favoring lemurs. Our results on the effect of socio-ecological factors and parasite specific richness on the VI support these findings. It has been observed that the fixed effect of polyparasitism has a negative impact on the VI, as well as the interaction of the factors favoring sex and year, sex, and period respectively. *Microcebus rufus* individuals infected with several species of parasites are less bulky and this is more noticeable in females before and during the mating season. This also demonstrates that the multiple infections could have a direct consequence on the host by depleting fat reserves, since the VI reflects the deposition of subcutaneous fat. Our model suggests that *Microcebus rufus* females infected by multiple parasite species have a lower VI. It has been found that either these low VI individuals have an inability to overcome multiple infections, or that the harmful effects of multiple infections lead to poor condition (Rodriguez-Zaragoza 1994); however, the worsening body condition could have a direct effect on survival and reproduction (Coop & Holmes 1996; Murray et al. 1998), with females being especially affected. Thus, it seems that there is a threat to the continued viability of the population of *Microcebus rufus* in Ranomafana National Park, which could result in a decrease of the number of *Microcebus* captures in this park. In addition, analysis of the effects of socio-ecological factors and parasitic infection measures on CI gives us valuable information. The fixed effect of parasitic

abundance on CI (difference or residual of SMi on M), suggests that the more a *Microcebus* individual gains weight (positive CI), the greater the number of eggs or oocysts in the feces. For females in particular, positive CI was associated with higher numbers of eggs per gram of feces (EPG). This suggests that it is the females that are the reservoir and responsible for the high incidences of gastrointestinal parasite in the study site. This same result showed us that a healthy host promotes the parasite's development cycle, meaning there is host tolerance in parasite reproduction. This demonstrates that parasitic infections do not always lead to an immediate effect on the host (Bize et al. 2008; Seppälä et al. 2008). Rather, the effects may manifest in the long-term fitness reduction of the host (Willis & Poulin 1999). Although we could not detect any clinical signs of infection, parasite-host relationships that are initially commensal may later affect the body condition when intrinsic and ecological contributing factors cause increased stress.

In the light of this study, the importance of analyzing and examining the synergistic effects between favoring factors and parasite infection on body conditions deserves a great deal of attention in conservation. The multiple infections suffered by individuals in poor condition provide a very important source for the transmission of parasites. In summary, we have been able to show the mechanism of parasitic ecology of a species of nocturnal lemur.

REFERENCES

Aivelo, T. (2015). *Longitudinal Monitoring of Parasites in Individual Wild Primates. (Vol. 2).* University of Helsinki, 52pp. https://doi.org/10.13140/RG.2.1.2218.0563.

Altizer, S., C.L. Nunn & P. Lindenfors, (2007). Do threatened hosts have fewer parasites? A comparative study in primates. *Journal of Animal Ecology* 76: 304–314.

Alzaga, V., J. Vicente, D. Villanua, P. Acevedo, F. Casas & C. Gortazar (2008). Body condition and parasite intensity correlates with escape capacity in Iberian hares (*Lepus granatensis*). *Behavioral Ecology and Sociobiology* 62: 769–775. https://doi.org/10.1007/s00265-007-0502-3.

Atsalis, S. (1999). Seasonal fluctuation in body fat and activity levels in a rain-forest species of Mouse lemur, *Microcebus rufus*. International Journal of Primatology 20(6): 883–910. https://doi.org/10.1023/A:1020826502103.

Beldomenico, P.M. & M. Begon (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology and Evolution* 25(1): 21–27. https://doi.org/10.1016/j.tree.2009.06.015

Bize, P., C. Jeanneret, A. Klopfenstein & A. Roulin (2008). What makes a host profitable? Parasites balance host nutritive resources against immunity. *American Naturalist* 171(1):107–118; http://doi.org/10.1086/523943

Chapman, C.A., M.D. Wasserman, T.R. Gillespie, M.L. Speirs, M.J. Lawes, T.L. Saj & T.E. Ziegler (2006). Do food avalaibility, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology* 131(4): SN 1096–8644. https://doi.org/10.1002/ajpa.20477

Coop, R.L., & P.H. Holmes (1996). Nutrition and parasite interaction. *International Journal for Parasitology* 26, 8–9:951–962. https://doi.org/10.1016/S0020-7519(96)80070-1

Dryden, M.W., P.A. Payne, R. Ridley & V. Smith (2005). Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts. Spring *Veterinary Therapeutics* 6(1): 15–28.

Durden, L.A., S. Zohdy, J. Laakkonen (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). *Journal of Parasitology* 96(5): 874–878. https://doi.org/10.1645/GE-2512.1

Esch, G. & J.C. Fernandez (1993). *A Functional Biology of Parasitism: Ecological and Evolutionary Implications*. Chapman and Hall, London, 289pp.

Ezenwa, V.O., R.S. Etienne, G. Luikart, A. Beja-Pereira & A. E. Jolles, (2010). Hidden consequences of living in a wormy World: Nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *The American Naturalist* 176(5): 613–624. https://doi.org/10.1086/656496

Gillespie, T.R. (2006). Non-invasive Assessment of Gastrointestinal Parasite Infections in Free Ranging Primates. *International Journal of Primatology* 27: 1129. https://doi.org/10.1007/s10764-006-9064-x

Gillespie, T.R., E.C. Greiner & C.A. Chapman (2005). Gastrointestinal parasites of the colobus monkeys of Uganda. *Journal of Parasitology* 92: 569–573.

Gillespie, T.R. & C.A. Chapman (2008). Forest fragmentation, the decline of an endangered primate, and changes in host-parasite interactions relative to an unfragmented forest. *American Journal of Primatology* 70: 222–230.

Green, A.J. (2001). Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82(5):1473–1483. https://doi.org/10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2

Hochachka, V.W. & A.A. Dhondt, (2000). Density-dependent decline of host abundance resulting from a new infectious disease. Proceedings of the National Academy of Science 97: 5303–5306.

Hudson, P.J., A.P. Dobson, & D. Newborn (1998). Prevention of population cycles by parasite removal. *Science* 282: 2256–2258.

Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek & A.P. Dobson (2002). The Ecology of Wildlife Disease. Oxford University Press, 240pp.

Kappeler, P.M. & C.P. Van Schaik (2002). Evolution of primate social systems. *International Journal of Primatology*, 23(4): 707-740.

Kappeler, P.M., S. Cremer & C.L. Nunn (2015). Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies. Philosophical Transaction of the Royal Society B: *Biological Sciences* 370(1669) https://doi.org/10.1098/rstb.2014.0116

Kessler, S.E., U. Radespiel, K. Schaber & C. Strube (2015). Tiny samples from tiny lemurs: methodological considerations for endoparasite analyses in mouse lemurs, pp210–219. In: Lehman S.M., Radespiel U., Zimmermann E. (eds.). *Biology, Behavior, and Conservation Biogeography of the Cheirogaleidae.* Cambridge University Press, Cambridge, 570pp.

Labocha, M.K., H. Schutz, & J.P. Hayes (2014). Which body condition index is best? (June 2013): 111–119. https://doi.org/10.1111/j.1600-0706.2013.00755

Loudon, J., M. Howell & A. Fuentes (2006). The importance of integrative anthropology: a preliminary investigation employing primatological and cultural anthropological data collection methods in assessing human-monkey co-existence in Bali, Indonesia. *Ecological and Environmental Anthropology* 2(1): 2–13.

Martin, L.B., A. Scheurlein II & M. Wikelski, (2003). Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London, B* 270:153–158. https://doi.org/10.1 098/rspb.2002.2185

May, R.M. (1988). Conservation and disease. *Conservation Biology* 2(1): 28–30. https://doi.org/10.1111/j.1 523-1739.1988.tb00332.x

Munyeme, M., H.M. Munang'andu, J.B. Muma, A.M. Nambota, D. Biffa & V.M. Siamudaala (2010). Investigating effects of parasite infection on body condition of the Kafue lechwe (*Kobus leche kafuensis*) in the Kafue basin. BMC Research Notes 3: #346. https://doi.org/10.11 86/1756-0500-3-346

Murray, D.L., L.B. Keith & J.R. Cary (1998). Do parasitism and nutritional status interact to affect production in snowshoe hares? *Ecology* 79:

1209–1222.

Neuhaus, P. (2003). Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). *Biology Letters* 270(2): 213–215. https://doi.org/10.1098/rsbl.2003.0073

Peig, J., A.J. Green & C. Ame (2009). New perspectives for estimating body condition from mass / length data: the scaled mass index as an alternative method, (May). https://doi.org/10.1111/j.1600-0706.2009.17643

Radespiel U., K. Schaber & S.E. Kessler (2015). Variations in the excretion patterns of helminth eggs in two sympatric mouse lemur species (*Microcebus murinus* and *Microcebus ravelobensis*) in northwestern Madagascar: 941–954.

Rafalinirina, A.H., T. Aivelo, J. Randrianasy & P.C. Wright (2015). Comparison of parasitic infections and body condition in rufous mouse lemurs (*Microcebus rufus*) at Ranomafana National Park, southeast Madagascar. *Madagascar Conservation & Development* 10(2): 60–66. https://doi.org/10.4314/mcd.v10i2.4

Raharivololona, B.M. (2009). Parasites gastro-intestinaux de *Microcebus murinus* de la forêt littorale de Mandena, Madagascar. *Madagascar Conservation & Development* 4: 52-62.

Rodriguez-Zaragoza, S. (1994). Ecology of free-living amoebas. *Crit Rev Microbiol* 20: 225–241.

Sánchez-Villagra M.R., T.R. Pope & V. Salas (1998). Relation of intergroup variation in allogrooming to group social structure and ectoparasite loads in red howlers (*Alouatta seniculus*). *International Journal of Primatology* 19(3): 473-491.

Schwitzer N, D. Clough, H. Zahner, W. Kaumanns, P.M. Kappeler & C. Schwitzer (2010). Parasite prevalence in blue-eyed black lemurs *Eulemur flavifrons* in differently degraded forest fragments. *Endangered Species Research* 12: 215–225.

Seppälä, O., K. Liljeroos, A. Karvonen & J. Jokela (2008). Host condition as a constraint for parasite reproduction. *Oikos* 117(5): 749–753; https://doi.org/10.1111/j.0030-1299.2008.1 6396.x

Sheldon, B.C. & S. Verhulst (1996). Ecological immunity: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 1: 317–321.

Silk, J.B. (1986). Social behavior in evolutionary perspective, pp318–329. In: Smuts, B.B., D.L. Cheney, R.M. Seyfarth, R.W. Wrangham & T.T. Struhsaker (eds.). *Primate Societies*. University of Chicago Press, Chicago, xi+578pp.

Stevenson, R.D. & W.A.Jr. Woods (2006). Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology* 46: 1169–1190.

Tompkins, D.M., A.M. Dunn, M.J. Smith & S. Telfer (2011). Wildlife diseases: from individuals to ecosystems. *Journal of Animal Ecology* 80(1): 19–38. https://doi.org/10.1111/j.1365-2656.2010.01742.x

Wilford, O. (1986). *Animal Parasites: Their Life Cycles and Ecology*. Dover Publications, New York, 564pp.

Willis, C. & R. Poulin (1999). Effects of the tapeworm *Hymenolepis diminuta* on maternal investement in rats. *Canadian Journal of Zoology* 77(6): 1001–1005. https://doi.org/10.11 39/z99-075

Wright, P. C. & B.A. Andriamihaja (2002). Making a rain forest national park work in Madagascar: Ranomafana National Park and its long-term research commitment, pp112–136. In: Terborgh, J., C. van Schaik, M. Rao & L. Davenport (eds.). *Making Parks Work: Strategies for Preserving Tropical Nature* Island Press, Washington D.C., 511pp.

Wright, P.C. (1992). Primate ecology, rainforest conservation and economic development: Building a national park in Madagascar. *Evolutionary Anthropology* 1(1): 25–33. https://doi.org/10.1002/evan.1360010108

Wright, P.C., S.J. Arrigo-Nelson, K.L. Hogg, B. Bannon, T.L. Morelli et al. (2009). Habitat disturbance and seasonal fluctuations of lemur parasites in the rainforest of Ranomafana National Park, Madagascar, pp 311–330 In: Huffman, M.A. & C.A. Chapman (eds.) *Primate Parasite Ecology: The Dynamics and Study of Host-Parasite Relationships*. Cambridge University Press, London, 548pp.

Zuur, A.F., E.N. Leno & C.S. Elphick (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1: 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

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Appendix 1. Result of PCA for the calculation of VI.

Total variance explained

Component	Proper initial value			Sum extracted from the square changes		
	Total	% of variance	% cumulative	Total	% of variance	% cumulative
1	2.45	48.32	48.32	2.42	48.32	48.32
2	0.94	18.83	67.16			
3	0.60	11.79	78.94			
4	0.56	11.25	90.19			
5	0.49	9.81	100.00			

Extraction methods: Principal component analysis.

Component matrix

	Component [a]
	1
Circumference of the base of tail	0.74
Circumference of the mid tail	0.55
Circumference of thigh	0.69
Circumference of the biceps	0.74
Body volume	0.75

Extraction method: Principal component analysis.

a. component extracted

Appendix 2. Mean eggs per gram of feces for Strongyloides and Hymenolepis, Mean of oocyst per gram of feces for Coccidia, mean of lice for ectoparasite, mean parasite species richness.

		***Strongyloides* sp.**	***Hymenolepis* sp.**	***Lemurpediculus verruculosus***	**Coccidia**	**PSR**
	N	Mean (min–max)	Mean (min–max)	Mean (min–max)	Mean (min–max)	Mean (min–max)
Sex						
F	87	278	13	3	11	1
		(0–4388)	(0–247)	(0–90)	(0–580)	(0–4)
M	**117**	**287**	**78**	**21**	**29**	**3**
		(0–3900)	(0–1000)	(0–145)	(0–950)	(0–5)
Site						
Campsite	71	242	24	5	6	2
		(0–2660)	(0–965)	(0–70)	(0–160)	(0–4)
Talatakely	119	256	**71**	**19**	10	2
		(0–3900)	(0–1000)	(0–145)	(0–245)	(0–4)
Ranofady	**14**	**724**	4	1	**199**	2
		(0–4388)	(0–50)	(0–6)	(0–950)	(0–5)
Period						
1	62	80	53	12	2	2
		(0–556)	(0–965)	(0–120)	(0–20)	(0–4)
2	**65**	**251**	**66**	**17**	**8**	**2**
		(0–1259)	(0–1000)	(0–145)	(0–160)	(0–4)
3	77	465	36	10	48	2
		(0–4388)	(0–518)	(0–140)	(0–950)	(0–4)
Year of study						
2012	120	155	80	16	8	2
		(0–2660)	(0–1000)	(0–145)	(0–160)	(0–4)
2013	47	239	0	13	3	1
		(0–1242)	0	(0–120)	(0–87)	(0–3)
2015	**37**	**754**	16	2	88	2
		(0–4388)	(0–217)	(0–40)	(0–950)	(0–5)

Min - minimum value | max - maximum value | PSR - Parasite Species Richness | period1: before mating season | period2: during mating season | period3: after mating season.

Appendix 3. Result of PCA for the parasites abundance collinear.

Total variance explained						
Component	Proper initial value			Sum extracted from the square changes		
	Total	% of variance	% cumulative	Total	% of variance	% cumulative
1	2.32	57.96	57.96	2.32	57.96	57.96
2	0.71	17.71	75.67			
3	0.59	14.76	90.44			
4	0.38	9.56	100.00			
Extraction methods : Principal component analysis..						

Component matrix [a]	
	Component [a]
	1
Oocyste per gram of feces for Coccidia	0.69
Eggs per gram of feces for *Strongyloides*	0.78
Eggs per gram of feces for *Ascaris*	0.75
Eggs per gram of feces for *Trichuris*	0.82

Extraction method: Principal component analysis.

a. component extracted.

Appendix 4. Parameters estimated from GLMM to evaluate the variation of the Volume Index in *Microcebus rufus*.

Terms of the model	coefficient	SE	t	p
Volume Index				
Constant	0.32	0.35	0.92	0.361
2012	-0.44	0.40	-1.10	0.273
2013	-1.09	0.55	-2.00	**0.048**
2015 (reference year)	0			
Female	0.50	0.42	1.19	0.236
Male (reference sex)	0			
Period 1	0.70	0.42	1.67	0.096
Period 2	0.23	0.46	0.49	0.623
Period 3	0			
Campsite	0.46	0.50	1.02	0.311
Ranofady	-1.12	0.39	-2.85	**0.005**
Talatakely	0			
PSR	-0.18	0.07	-2.56	**0.012**
Parasite abundance	-0.00	0.11	-0.04	0.971
2012*F	0.07	0.50	0.15	0.884
2012*M	0			
2013*F	1.26	0.54	2.32	**0.022**
2013*M	0			
2015*F	0			
2015*M	0			
2012*period 1	0.14	0.38	0.36	0.718
2012*period 2	0.18	0.47	0.39	0.700
2012*period 3	0			
2013*period 1	0			
2013*period 2	0.16	0.56	0.29	0.773
2013*period 3	0			
2015*period 2	0			
2015*period 3	0			
2012*Campsite	-0.03	0.32	-0.11	0.915
2012*Talatakely	0			
2013*Campsite	0			
2013*Talatakely	0			
2015*Ranofady	0			
2015*Talatakely	0			
F*period 1	-1.05	0.36	-2.95	**0.004**
F*period 2	-0.92	0.32	-2.86	**0.005**
F*period 3	0			
M*period 1	0			
M*period 2	0			
M*period 3	0			

SE: Standard Error, *t*: Student test, *p*: probability, F: female, M: male, period 1: before mating season, period 2: during mating season, period 3: after mating season, PSR: Parasite Species Richness.

Appendix 5. Parameters estimated from GLMM to assess body condition index (CI) variation in *Microcebus rufus*.

Terms of the model	coefficient	SE	t	p
Condition Index				
Constant	3.09	1.01	3.07	**0.003**
2012	-3.75	1.14	-3.30	**0.001**
2013	-4.39	1.29	-3.40	**0.001**
2015 (reference year)	0			
Femelle	-0.73	1.25	-0.58	0.562
Male (reference sex)	0			
Period 1	-0.13	0.67	-0.20	0.840
Period 2	-0.74	1.32	-0.56	0.578
Period 3	0			
Campsite	-0.64	1.07	-0.60	0.550
Ranofady	-6.45	1.19	-5.44	**0.000**
Talatakely	0			
PSR	0.03	0.13	0.23	0.822
Parasite abundance	0.60	0.29	2.04	**0.043**
2012*F	0.66	1.48	0.45	0.655
2012*M	0			
2013*F	3.69	1.52	2.43	**0.016**
2013*M	0			
2015*F	0			
2015*M	0			
2012*period 1	0.03	0.60	0.05	0.957
2012*period 2	0.91	1.32	0.69	0.491
2012*period 3	0			
2013*period 1	0			
2013*period 2	1.03	1.41	0.73	0.467
2013*period 3	0			
2015*period 2	0			
2015*period 3	0			
2012*campsite	0.78	0.52	1.49	0.138
2012*Talatakely	0			
2013*campsite	0			
2013*Talatakely	0			
2015*Ranofady	0			
2015*Talatakely	0			
F*period 1	-0.46	0.54	-0.85	0.399
F*period 2	-0.08	0.53	-0.15	0.884
F*period 3	0			
M*period 1	0			
M*period 2	0			
M*period 3	0			

SE - Standard Error | *t* - Student test | *p* - probability | F - female | M - male | period 1 - before mating season | period 2 - during mating season | period 3 - after mating season | PSR - Parasite Species Richness.

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