Research article

Gastrointestinal parasites and ectoparasites in wild Javan slow loris (*Nycticebus javanicus*), and implications for captivity and animal rescue

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Abstract

Javan slow lorises (Primates: *Nycticebus javanicus*) are heavily threatened by anthropogenic disturbance and the illegal animal trade, both of which may impact parasite loads. They are also venomous, which may have evolved to help reduce parasite burdens. We present analyses of faecal samples for evidence of gastrointestinal parasites and data based on ectoparasite inspections from wild Javan slow loris collected over a 13-month period. We sampled 21 *N. javanicus* for parasites at Cipaganti, Garut District, West Java, Indonesia. We found most individuals were infected with gastrointestinal parasites. We report hookworm (*Necator* spp.) – eggs and adults; pinworms (*Lemuricola* spp.) – eggs and adults; and *Trichostrongylus* – eggs and adults. We found evidence for only one ectoparasite infection in 61 captures, this being a rash on one adult male. Although we could not identify the parasite, it had close resemblance to a skin mite species. Prevalence and intensity of infections with *Lemuricola* spp. were not related to weather periods or sex, but showed a tendency of fewer infections in immature slow lorises. The role of slow loris venom in the defence of ecto- and endoparasites is discussed. We emphasise the importance of natural diet to ensure an appropriate immune reaction including venom sequestering in rescue centres. *Lemuricola* spp. have not been documented for any other slow loris species which emphasises the danger of not recognising taxonomic differences and geographical distribution in reintroduction planning.

Introduction

Parasite load may affect the fitness of the host, influencing the survival and reproduction of the infected individuals (Behnke 1990; Despommier et al. 1995; van Vuren 1996, Hilser et al. 2014). Threatened species are characterised by small populations and often live in disturbed and fragmented habitat. The chronic stress they may experience caused by low food availability, restricted ranging opportunities and possibly crowding effects through locally high population sizes, makes them more susceptible to parasites and infectious diseases (Lyles and Dobson 1993; Wright et al. 2009; Arroyo-Rodríguez and Dias 2010; Schwitzer et al. 2010). Animals that are subject to human exploitation or intervention, such as wildlife trade and translocations, may exhibit similarly high levels of stress (Clark et al. 2008; Dickens et al. 2010). Parasite infections and a low immune system due to chronic stress can be the last trigger leading to deteriorating health (Glaser and Kiecolt-Glaser 2005; Clark et al. 2008; Coe 2011). Inbreeding caused by fragmentation has been suggested to be associated with higher parasite prevalence (Schad et al. 2005). Although fragmentation may decrease the diversity of parasite species (Anderson and May 1982), human encroachment results in the sharing of habitat and increased interactions between humans and primates, and thus increases anthropozoonotic transmission (de Thoisy 2001; Graczyk et al. 2001)

Baseline patterns of parasite infection in wild populations are important to detect increased parasite loads and to understand which parasites are naturally found in slow loris populations, and which have been acquired due to proximity to humans. Animals may not be immune to the latter and infections may have serious health consequences. Thus, parasitology is of considerable importance with regards to successful conservation management, including small population

management or planning for rehabilitation and reintroduction of animals (Cowlishaw and Dunbar 2000; Daszak et al. 2000; Foitova et al. 2009).

Slow lorises are nocturnal arboreal primates inhabiting South-East Asia. Slow lorises are specialised exudate feeders and live in groups of one male, one female and offspring (Nekaris and Bearder 2011). All eight species are severely threatened by habitat loss and wildlife trade for traditional medicine and pets, with the Javan slow loris (*Nycticebus javanicus*) being assessed as Critically Endangered on the IUCN Red List (Nekaris et al. 2013a). Although slow lorises are legally protected by national law, they are the most traded primate species in Indonesia (Shepherd 2010). Slow lorises are venomous; animals produce a toxic liquid by combining brachial gland exudates with saliva (Hagey et al. 2007). It has been suggested that anointment with this secretion or its digestion might serve as an ectoparasite defence (Nekaris et al. 2013b). As many animals exploit the bioactive properties of secondary plant metabolites to treat against bacterial or parasitic infections (Forbey et al. 2009), venom may also assist in the defence against endoparasites.

A part of slow loris venom may be sequestered from secondary plant metabolites such as those found in gum or noxious arthropods (Rode-Margono 2015). If venom is sequestered, variations in food lead to variations in toxicity (Dumbacher et al. 2009), and – as is the case in poison dart frogs (Daly et al. 1992) – the lack of a natural diet in captivity may decrease the effectiveness of venom and consequently cause higher parasite loads in slow lorises.

Here we describe endo- and ectoparasites exhibited by wild Javan slow lorises and examine the effects of season, sex and age on the prevalence and intensity of one of the endoparasite species detected, the pinworm *Lemuricola* spp. (Chabaud and Petter 1959, family Oxyuridae). We furthermore test the hypothesis that the presence of gum (containing secondary plant metabolites) and/or caterpillars (as potentially noxious arthropods) has an effect on the prevalence and intensity of pinworms. We use our results to formulate recommendations for the husbandry and release schemes of rescue centres.

Methods

Study site
From April 2012 to June 2013 we conducted our study in Cipaganti, a small agricultural area near Garut, West Java, Indonesia (S7°6′6–7°7′0 and E 107°46′0–107°46′5). The study site is not protected, but lies at the foothills of Mount Papandayan, a recognised Nature Reserve (*cagar alam*). The study site consists of a mosaic of agricultural gardens and small forest and bamboo patches, locally referred to as *talun*. The research area was 2.2 km² and was located at 1200–1700 m asl. The climate in the area can be described as humid and tropical with two distinct weather periods, a rainy period from October to April and a dry period from May to September. The average daytime temperature ranged between 19 and 28° C, whereas the average night time temperature ranged between 10 and 22° C (Rode-Margono et al. 2014).

Sampling of parasites and food remains
We radio-tracked twelve slow lorises over the course of 14 months and re-captured all animals every three months for a health check and the collection of samples. We also captured nine additional animals for sampling purposes. Female adults weighed on average 884±61 g (840–974 g) and male adults 905±65 g (820–1025 g) (Rode-Margono 2015). We collected faecal samples (> 2 g) from all animals and stored them in ethanol. We diluted the faeces with water and thoroughly examined them with the naked eye for the presence of adult pinworms, that are easily visible, and food remains (gum, arthropods, arthropod wings, caterpillars, seeds,

bark). If necessary, we used a microscope (total magnification 50x, 100x and 200x) for confirmation. We defined prevalence of parasites and different food items by the number of individuals of a host species infected divided by the number of hosts examined, and intensity by the number of individuals of a particular parasitic species in each infected host (Stuart 1995). Although the number of faecal eggs does not necessarily reflect the severity of infestation (Gillespie 2006) we decided to report the number of adult worms in the faeces.

In addition to this, in May and June 2012 I examined eight samples in more detail, following a wet lab protocol based on Gillespie (2006) and Hilser (pers. comm.). We subdivided the fresh samples stored one part in acetic acid–formalin solution with triton X-100. From these samples we placed approximately 1 g of faeces into a 15 ml centrifuge tube using a wooden applicator stick. The tube was filled two thirds of the way up with de-ionised water and homogenised with the same wooden applicator stick. Then the wooden applicator stick was removed and the tube centrifuged for 10 min at 1800 rpm. The supernatant was decanted and the faeces re-suspended in sodium nitrate ($NaNO_3$-) solution with a specific gravity of 1.18–1.20. The faeces were mixed with the solution and poured through a sieve into a 15 ml centrifuge tube and were then spun for 5 min to improve separation between faecal matter and parasites. The tube was filled until a slightly positive meniscus formed, the coverslip was placed on the tube and the tube was allowed to stand for 20 min. The coverslip was removed and placed on a labelled glass slide for microscopic examination. We examined the slides under a total magnification of 100x; when necessary 400x was used to confirm diagnosis (Dryden et al. 2005; Gillespie 2006). Strongyloid eggs were identified by their size, colour, shape and morula aspect. Nematodes were identified following Do (2009), Huffman and Chapman (2009) and Gillespie et al. (2010). Photos of parasites were sent to Dr Ivona Foitova (Orangutan Health Project) and Dr Lynda Gibbons for confirmation. During every capture, we thoroughly examined the fur for ectoparasites, parting the hair and especially checking ectoparasite-prone body parts such as ears, face and anogenital area.

Data analysis
Due to small sample sizes and non-normal distribution of data we used descriptive and non-parametric statistics. Confidence limits are given by the standard deviation of the mean. We used one-sided Fisher's Exact Tests to investigate a relationship between pinworm presence in the sample and season, sex, age, presence of gum and presence of caterpillars in the sample (Field 2009). We set the significance level at P=0.05. We categorised the faecal amount into small (= 1), medium (= 2) and large (= 3). The faecal amount index per individual was calculated by dividing the sum of the faecal amounts by the number of captures.

Results

We collected 43 faecal samples from different captures of 21 individuals. Seven of eight samples that we examined using sodium nitrate flotation were infected with gastrointestinal parasites (Table 1). All these parasites were nematodes. In the smear samples and macroscopic examination we found hookworm *Necator* spp. (family Ancylostomatidae), eggs (65x40 μm) and adults (10 mm); pinworms *Lemuricola* spp. (family Oxyuridae), eggs (60x25 μm) and female adults (11 mm); and *Trichostrongylus* spp. (family Trichostrongylidae), eggs (63x40 μm) and adults (8 mm). None of the samples showed blood or mucus.

The macroscopic examination of all 43 samples revealed a total pinworm prevalence of 69.8% with an average intensity of 3.0±4.3 worms, range 0–21 worms. Details of animals with at least three samples from different captures are shown in Table 2. The faeces

Table 1. Results of sodium nitrate flotation of faecal samples (E=eggs, A=adults) of eight wild Javan slow lorises captured in an agricultural area in West Java in May and June 2002.

Sex	Age	Weight (g)	Hookworm	*Trichostrongylus*	Pinworm	Mites
Male	Adult	808		E+A	E+A	
Male	Adult	820	A			
Male	Adult	895		E		
Male	Adult	898	E+A			A
Male	Adult	1025	E		E+A	
Male	Sub-adult	757	A		E+A	
Female	Adult	893	A	E		
Female	Su-adult	850				

of one female loris had a pinworm full of eggs in the dry weather period, and another sample from a female showed many (>30) pinworms of about 2–3mm as well as six pinworms of about 1 cm in the wet weather period.

We examined 43 different faecal samples and found arthropod remains in 95%, wings in 72%, gum in 70%, bark in 67%, seeds in 40%, and caterpillars in 28% of the samples. Samples with arthropod remains contained a median of 11 countable arthropod pieces (range 1–52), samples with wings a median of two (range 1–14) and samples with seeds a median of two (range 1–64). We counted seven different types of seeds. However, the taxa of fruit plants and arthropods could not be identified. None of the variables tested here had a significant relationship with pinworm presence in the sample (Fisher's Exact Test: sex P=0.204; season P=0.540; age P=0.052; caterpillar P=0.187; gum P=0.277; Fig. 1). For worm intensity, none of the variables were significant either (Mann–Whitney U-Test: sex U=288, P=0.131; season U=257, P=0.515; age U=110.5, P=0.068; caterpillar U=244.5, P=0.072; gum U=185.5 P=0.880). Age approached significance in both tests, with a lower prevalence and intensity for younger animals.

Only once in 61 captures of 21 individuals over 14 months did we detect evidence of ectoparasites. An adult male exhibited an extensive skin rash on the throat, shoulder and upper chest in the dry weather period. The animal lost its fur on the infected area and the skin looked dry and scabby. We took a skin scrape and found an unidentified ectoparasite at a magnification of 40x (Figure 2).

This parasite was 52 μm in size. Although we could not identify the species, based on the presence of the rash and the shape of the parasite, it might be a skin mite species. We found no eggs, larvae, nymphs or signs of reproduction by the skin mites. At the next health check after three months, the rash had healed completely. No signs of ectoparasites were found on any other Javan slow loris and in all cases animals had very healthy fur condition.

Discussion

We identified three different gastrointestinal parasites in wild Javan slow lorises, which were all nematodes: hookworm *Necator* spp., pinworms *Lemuricola* spp. and *Trichostrongylus* spp. These parasites are common amongst primates (Munene et al. 1998; Gillespie et al. 2005 a,b; Chapman et al. 2005; Ekanayake et al. 2006; Foitová et al. 2009). Other primates exhibiting these parasites include the toque macaque (*Macaca sinica*), olive baboons (*Papiocyanocephalus anubis*), vervet monkeys (*Cercopithecus aethiops*), blue monkeys (*Cercopithecus mitis*), Delacour's langur (*Trachypithecus delacouri*) and chimpanzee (*Pan troglodytes schweinfurthii*).

Nematodes have been identified in slow lorises before, including *Trichuris, Strongyloides, Strongylus, Gongylonema, Oxyuris, Enterobius, Physaloptera, Filaria, Spirura, Microfilaria, Breinlia, Pterygodermatides* (Sutherland-Smith and Stalis 2001;

Table 2. Prevalence of the pinworm *Lemuricola* spp. (proportion of infected samples) and intensity (mean number of parasites found in the infected hosts) of six adult wild Javan slow lorises with at least three faecal samples from different captures. The faecal amount index (FAI) was calculated by dividing the sum of small (=1), medium (=2) and large (=3) samples by the number of captures.

Name	Sex	Age	Weight	N	Intensity	sd	Min	Max	Prevalence	FAI
MO	male	adult	915	5	4.6	3.4	1	9	1.00	2.20
AZ	male	adult	870	5	1.2	1.6	0	4	0.60	2.25
YO	male	sub-adult	800	4	1.5	1.7	0	3	0.50	2.25
EN	female	adult	765	3	5	4.6	0	9	0.67	2.00
TE	female	adult	831	3	3.3	3.4	1	5	1.00	2.33
CH	female	adult	920	3	2.3	3.2	0	6	0.67	1.33
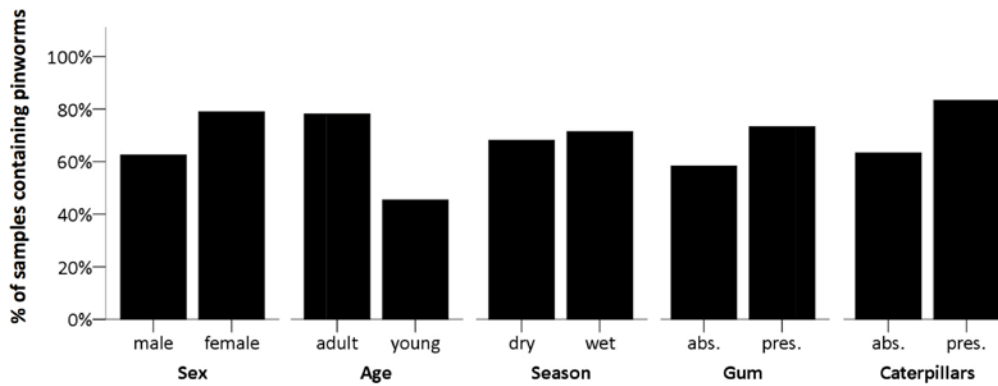
Figure 1. Proportions of samples that contained pinworms for different sexes, seasons, age classes, and whether samples contained caterpillar and gum remains. N = 43.

Streicher 2004). Except for *Pterygodermatides*, which causes fatal anaemia (Tuggle et al. 1984; Sutherland-Smith and Stalis 2001), most infections seem to be asymptomatic. Setyorini and Wirdateti (2005) found the nematodes *Syphasia* sp., *Enterobius* sp. (Oxyuridae) and *Ricyularia* sp. (Rictularidae), as well as a tapeworm (Platyhelminth, Cestoda: Cyclophillidae) and thorny-headed worms (Acanthocephala) in greater slow lorises (*Nycticebus coucang*) confiscated from the pet trade. Only a few taxa of protozoan parasites, cestodes, trematodes or acantocephala have been reported for slow lorises (*Giardia*, *Trichomonas*, *Cryptosporidia*, *Trypanomsoma*, *Hymenolepis*, *Phaneropsulus*, *Echinorhynchus*) (Sutherland-Smith and Stalis 2001). Except for Streicher (2004), who worked on pygmy lorises (*Nycticebus pygmaeus*) arriving at a rescue centre, all accounts are from captive animals. Thus, our records of parasites are the first for wild slow lorises.

In 69.8% of 43 faecal samples we found adult pinworms (*Lemuricola* spp.). Existing parasite studies of captive slow lorises have never before reported *Lemuricola* spp. In pygmy lorises, Streicher (2004) found a related species of the same family, Oxyuridae (*Enterobius* spp.) in the faeces and around the anus of some animals that arrived in the rescue centre. Sutherland-Smith and Stalis (2001) found *Enterobius* during one of 16 pygmy loris health checks but none in 29 health checks of *Nycticebus coucang*.
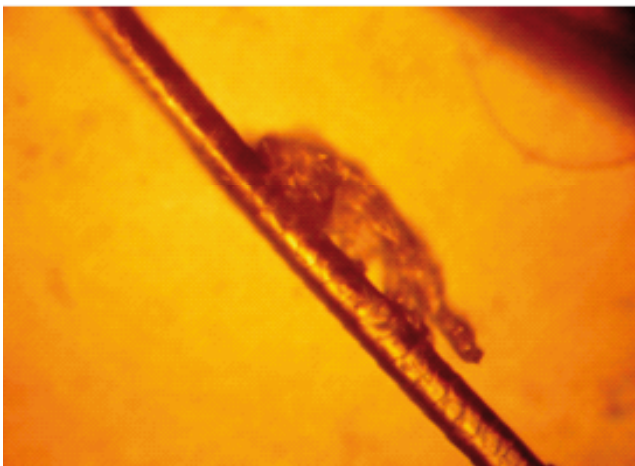
Less than 10% of rainforest remains in Java (Lavigne and Gunnell 2006). Due to the associated stress level caused by food availability, ranging patterns, population sizes and anthropozoonotic transmission of pathogens, habitat quality decreases and habitat disturbance increases the risk of parasite infections and is associated with an overall greater prevalence of parasite infection (Lyles and Dobson 1993; Chapman 2005; Gillespie et al. 2005b, 2008; Wright et al. 2009; Schwitzer et al. 2010). Our field site, Cipaganti, is subject to high anthropogenic modification; it consists of a mosaic of agricultural fields, interspersed with single trees and small forest and bamboo patches (Rode-Margono et al. 2014). Eighty-two percent of primate parasites are transmitted via faecal–oral transmission (Hopkins and Nunn 2007). Local farmers work in the fields in our study site every day, sometimes bringing cattle, and all eating, drinking and defaecating daily. *Trichostrongylus* spp. do occur in animals such as goats that are commonly farmed in Indonesia (Rushton et al. 2002), *Necator* (*americanus*) affects humans (Bethony et al. 2006), and *Lemuricola* spp. may be hosted by domestic animals (Loudon et al. 2006). Due to the low tree density at this site, Javan slow lorises frequently have to come down to the ground to cross fields (Rode-Margono et al. 2014), suggesting a higher risk of being contaminated. If hosts have adequate energy reserves or nutrient supplies parasite infection may have little effect on them (Chapman et al. 2005; Gillespie 2006), but disturbed habitat may force animals to feed on a lower quality or quantity of food, and may lead to compromised body condition and reproductive status when parasites inflict substantial energetic costs. We had no reason to believe that lorises were restricted in their nutrition, as all lorises captured were in good body condition. Due to the lack of information on parasite prevalence and intensity in (Javan) slow lorises, we cannot conclude whether the animals in this study have higher or lower parasite burdens than normal. Although we conducted no special veterinary checks, animals seemed to be asymptomatic. For instance, none of our animals showed blood or mucus in the faeces.

Although several macroscopic ectoparasites have been reported for lorises, such as lice, ticks and fleas (Wiens 2002; Streicher 2004), compared to other primates members of the Lorisidae family are remarkably ectoparasite-free (Rode and Nekaris 2012). Only one of nine wild studies across six taxa found a small amount of ticks in all animals during the wet weather period (Wiens 2002; Nekaris et al. 2013b). In accordance with our results, all other studies rarely or never found any ectoparasites (reviewed by Rode and Nekaris 2012; Nekaris et al. 2013b).

Figure 2. Ectoparasite of an adult male Javan slow loris, total magnification 100x

Feeding on arthropods can be easily missed in observations of wild animals, due to dense habitat, distance to the animal, small food items and rapid feeding movements. Although most studies using direct observations suggest low frequencies of arthropod feeding for most species of slow lorises (below 10% for all species except *N. pygmaeus*, reviewed in Rode-Margono et al. 2014), more recent work indicates a much higher proportion, e.g. that more than a third of feeding time in Javan slow lorises is spent on arthropods (F. Cabana, pers. obs.). Faecal analysis in our study indicated that arthropods, including caterpillars, are among the most frequently ingested food items. Wiens et al. (2006) found a similarly high prevalence of 91.5% arthropods in greater slow lorises. Slow lorises are specialised exudate feeders (Nekaris et al. 2010; Nekaris 2015). Extensive gum feeding is confirmed in all studies on feeding in wild slow lorises (reviewed in Rode-Margono et al. 2014). While gouging holes to stimulate gum flow, slow lorises also ingest bark. Although the prevalence of fruits was comparatively small, we counted seven different seed types, which may indicate that slow lorises consume at least a certain variety of fruits. Nectar and sap are not traceable but seem to be important diet components as well (Wiens et al. 2006; Moore & Nekaris 2011).

One of the ecological functions of slow loris venom may be parasite defence (Nekaris et al. 2013b), and it is possible that the venom interrupts the parasite's lifecycle by killing parasites when they are in the mouth or on the skin and thus preventing infection. Animals secrete the venom from the brachial gland (Hagey et al. 2007). By licking their own brachial gland regions and wiping these glands against their heads, lorises combine fluid from the brachial gland with saliva (Hagey et al. 2007). Slow lorises exhibit solitary torpor and infant parking in the wild (Wiens and Zitzmann 2003; Xiao et al. 2010; Nekaris and Bearder 2011). Anointment of their own or their infant's fur with a secondary compound of the venom could be crucial in their health maintenance (Nekaris et al. 2013b). Indeed, Nekaris et al. (2013b) applied the combined venom exudates and saliva onto 12 (comparatively large) leeches that all died within minutes. Subsequent experiments have shown that loris venom can kill a wide variety of arthropods (Grow et al. 2014). The tendency of immature animals to exhibit a lower prevalence and intensity of pinworms shown in this study supports the anti-parasite function of anointing infants with venom, assuming that they would ingest the venom during subsequent autogrooming. Although based on a low sample size, immature animals tended to exhibit a lower prevalence and intensity of pinworms. It is possible that frequent anointment by their mothers to prevent infections during parking causes lower parasites loads. Alternatively, immature animals themselves may possess more effective venom as their bites have been reported to have serious effects (Madani and Nekaris 2014). Although, due to lack of comparative data, we cannot conclude that endoparasite prevalence in this study is high or low, ingested venom could also play a role in gastrointestinal parasite defence. Some insectivores (European water shrews *Neomys fodiens*, American short-tailed shrews *Blarina brevicauda*, Haitian solenodons *Solenodon paradoxurus*), or lizard species of the clade Toxicofera (e.g. monitor lizards *Varanus* spp.) use venom for digestive purposes and/or oral hygiene (Arbuckle 2009; Fry et al. 2009; Folinsbee 2013). Likewise, slow loris venom may kill certain life stages of parasites in the mouth or digestive tract.

Venom in slow lorises may be sequestered from secondary plant compounds and noxious arthropod prey. We could not find a significant relationship between the presence of gum or caterpillars and the presence of endoparasites. Again, this may be a reflection of the low sample size. Alternatively, it may also indicate that venom is not used to reduce endoparasites, or that it is not sequestered from dietary items eaten by slow lorises at our site.

Thorough health checks and risk assessments, especially in respect to parasites, are compulsory for all translocations of wild animals, including reintroductions following the confiscation of rescued animals (Leighton 2002; IUCN/SSC 2013). Our results could have implications for rescue centres that receive confiscated slow lorises. Poor treatment during trade means that slow lorises arrive in rescue centres in bad health condition, including potentially high stress levels and parasite burdens. Unlike in other primate species, where parasites may be seasonal (Semple et al. 2002), we found endoparasites in Javan slow lorises throughout the year, regardless of the weather period, whereas macroscopic ectoparasites were virtually absent. Wild slow lorises are known to consume various foods, particularly exudates, which cure human ailments and reduce parasite loads (Das et al. 2014). Slow lorises kept in rescue centres may lack dietary choices that would allow them to cope with parasites in the wild. Better mimicking wild diet may improve the welfare and treatment of captive lorises. The fact that we document *Lemuricola* spp. for the first time in slow lorises means that the Javan slow loris may have acquired some resistance to this parasite. Confusing species in captivity or poorly planned releases may transfer the parasites to more sensitive species. This emphasises how important the exact knowledge of slow loris taxonomy, the different species' geographic distribution and origin of confiscated animals are.

Acknowledgements

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