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Gastrointestinal parasitism of *Astrochelys radiata* turtles in Tsimbazaza Botanical and Zoological Park, Madagascar

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Abstract: *In Madagascar, the reptile is important in terms of species richness and represents a high rate of endemism up to 92%. The radiated turtle or Astrochelys radiata is one of five endemic species that are highly threatened due to illegal exploitation and habitat loss. Conserving the turtles in the Tsimbazaza Botanical and Zoological Park is one way of protecting the Astrochelys radiata species from human threats. But parasites could infest them, which is the reason for our study. The aim of this experiment was to find out about the types of parasites and the rate of infestation in these turtles. Twenty parasites were detected, of which the species belonging to the nematode class are the most common. All radiata turtles in the Tsimbazaza Botanical and Zoological Park are infested with parasites at different rates. In addition, a proposal for treatment and improvement of turtle breeding in captivity was put forward*

I. INTRODUCTION

The radiated tortoise or Astrochelys radiata (Shaw, 1802) is one of the five species of turtles endemic to Madagascar (Leuteritz et al., 2008; Pedrono, 2008). Since the 1970s, many threats to this species of turtle have led to the reduction and decline of their populations in Madagascar. Radiata turtles are subject to illegal exploitation (O'Brien et al., 2003; Leuteritz et al., 2005) and habitat loss (Mitchell and Klemens, 2000) due to human activities. A zoological park is an ex-situ conservation area for animal species that are threatened in their natural environment. The Tsimbazaza Botanical and Zoological Park is the best known in Madagascar, where *Astrochelys radiata* can be found. In this site, the turtles are protected from threats caused by man, but they could be affected by parasites that threaten their health.

Helminth parasites could affect turtles both in the wild and in captivity (Baker, 1978).

Parasitic studies of turtles are little studied in Madagascar compared with other reptiles. In addition, studies focus on population dynamics, distribution and reproduction of turtles (Leuteritz, 2002; O'Brien, 2002; O'Brien et al., 2003; Leuteritz et al., 2005; Leuteritz and Ravolanaivo, 2005). Parasites in Astrochelys radiata turtles are therefore less studied by researchers. There are fewer works concerning parasites in *Astrochelys radiata*, the

most recent of which is Étude des parasites gastro-intestinaux des testudines *Astrochelys radiata* (Shaw, 1802) et *Pyxis arachnoides* (Bell, 1827) dans le Parc National, published by Andriantsoa in 2013.
Therefore, a health study may be necessary to improve the health and longevity of Astrochelys radiata turtles. The aim of this study is to find out the types of gastrointestinal parasites and the rate of parasitic infestation in *Astrochelys radiata* turtles in Tsimbazaza Park.

II. Materials and methods

II.1. General aspects

The tortoise *A.radiata* owes its name to its carapace where yellow spots in the shape of rays can be found on a black background, hence the name radiated or star tortoise (Shaw, 1802).
This endemic tortoise of Madagascar, also known as the star tortoise *Astrochelys radiata*, belongs to the Testudinidae family, meaning that these animals can spend their entire lives on land.

II.2. Morphology

Males are bigger and fatter than females: the carapace of males measures between 28.5 and 39.5 cm, while that of females ranges from 24.5 to 35.6 cm. Males weigh an average of 6.7 kg and females 5.5 kg (Pedrono, 2008). However, the secondary sexual characteristics are also distinct: the tail is longer and wider in the male, with a larger anal opening and a concave plastron (Pedrono, 2008).
The tortoise is a reptile with a long lifespan that can reach a certain number of years (Glaw and Vences, 2007). Radiused turtles can live for around 137 years (Glaw & Vences, 2007). *Astrochelys radiata* had a lifespan of 188 years according to Pedrono (Pedrono, 2008).

II.3. Biological material

The turtle *Astrochelys radiata* is the subject of our study, more specifically its faeces. The Park's *Astrochelys radiata* turtle was chosen for this study for the purpose of analysing its health and monitoring its development in its conservation environment, where it is kept in captivity and its living conditions are unsatisfactory. This study will help to improve the husbandry of the site's turtles, which attract many visitors to the park. Historically, *Astrochelys radiata* species have been present in the park since 1927 according to information from the Wildlife Department, herpetology division PBZT in 2021.

II.4. Methods

Our research is based on three main methods: sedimentation (Boray (1969) and Rojo-Vázquez (2012)), flotation (Sloss et al., 1994) and Mac Master counting (Zajac et al, 2012).
This technique is derived from the modified flotation method.

II.5. Statistical analysis

Statistical analyses were performed using XLSTAT 2014 software for descriptive testing of the data.
The Chi Two Test was applied to compare the parasites detected between the flotation method and the sedimentation method. The Chi-square contingency test was used to analyse the relationship between parasites and turtle sex. Differences were considered significant at the 5% threshold (if p-value is less than alpa=0.5).
The correlation test of parasites with quantitative variables (Age and Weight) was carried out to study the link between these variables. Spearman's linear correlation test was used to analyse the relationship between parasite distribution and turtle age and weight. Spearman's correlation coefficient 'r' varies from -1 to 1, so the strength of the linear relationship will be stronger the closer the value of the coefficient is to +1 or -1, and weaker the closer it is to 0. If it is between 0.8 and 1 (in absolute value), the strength of the association between the two variables is strong; between 0.5 and 0.8, it is moderate; between 0.2 and 0.5, it is weak, and very weak below that.

II.6. Collecting faeces

- Age

According to Wilson and Tracy (2003), a new growth ring is formed every year. To easily determine the age of the turtles, the shells were washed at around 10 a.m. (light and at room temperature). The streaks were then counted using a loupe. The number of growth rings on the dorsal scale of the carapace indicates the age of the turtles (Castanet et Cheylan, 1979).

- Weighing

The turtles are weighed before the morning meals are distributed. The weight of each individual is recorded on a data recording sheet.

- Sexing

The sex of the tortoises is determined by the shape of the plastron. In males, the plastron is concave to facilitate mating; in females, however, the plastron is flat.

- Faeces

Faeces are collected in the morning between 7am and 2pm. Each individual is closely monitored. The faeces are stored in numbered and dated plastic jars. The material collected was immersed in 70° alcohol to preserve the physical properties of the parasite eggs (Daynès, 1964). Everything is put in a cooler and sent to the analysis laboratory.

III. Results

III.1. Sampling

During the field study, twenty-one radiata turtles were represented in the park, including 17 males and 4 females. The age of these turtles varies between 7 and 16 years for the males and 11 to 16 years for the females. Their weight varies from 0.7 to 11.9 kg. This Table 1 represents the data collected concerning the *Astrochelys radiata* tortoises preserved in the Botanical and Zoological Park of Tsimbazaza (BZPT).

Table 1: Data on the 21 radiata studied in the BZPT

Data	**Number**	**Weight (Kg)**			**Ages (year)**		
Sexe of turtle		**Min**	**Max**	**Average**	**Min**	**Max**	**Average**
Male	**17**	0,775	11,9	6,353	7	16	13
Female	**4**	7,4	8,6	7,9	11	16	13

III.2. Distribution of parasites in Astrochelys radiata turtles

This figure (Figure 1) shows us that all *Astrochelys radiata* are contaminated with parasites whose distribution varies between 2 and 10 parasites. At least two types of parasites were identified per turtle.

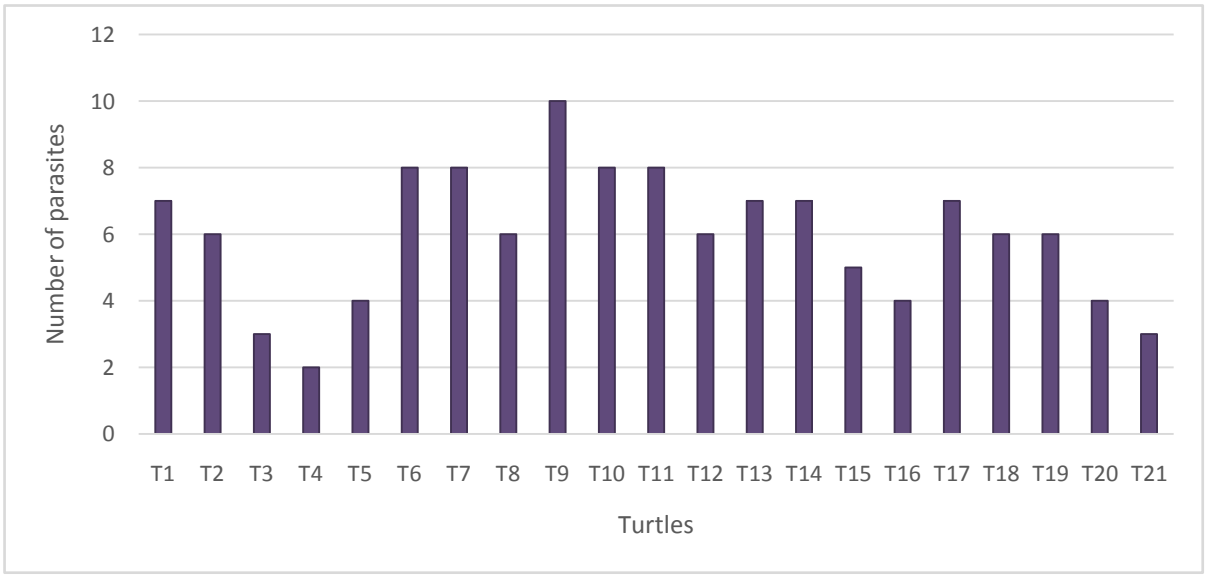

Figure 1: Distribution of parasites for each turtle

III.3. Coproscopic results

Twenty species of parasites were detected during microscopic observation. These 20 species are divided into 3 major classes: Nematode class, Protozoa class, Litostomatea class, the proportions of which are shown below (Figure 2), although six species were not identified. Nematodes were the most frequently represented parasitic class with a rate of 50%.

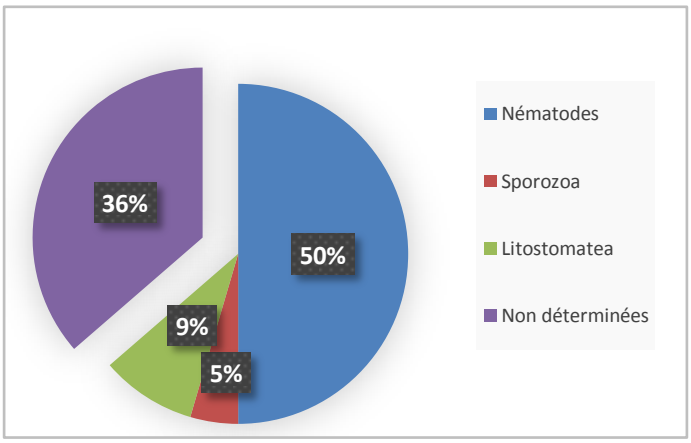

Figure 2: Distribution of parasites detected in *A.radiata*

As regards the order of the species identified, five orders were found: Oxyurida; Ascaridia, Strongilyda, Coccidia, Vestibuliferida (Figure 3).

Figure 3: Species of parasites detected in turtles: (A) *Oxyuris* ; (B) *Ascaridia*, (C) *Strongiloides*, (D) *Coccidi* sp, (E) *Balatidium* sp.

III.4. Comparison of parasites using the sedimentation and flotation methods

This table (Table 3) shows the number of parasites observed using the two methods: sedimentation and flotation. According to the Chi-square test, there is a significant difference between these two methods with a p-value < 0.0001, given that alpha= 0.05.

The parasites observed by the sedimentation method were more numerous than those observed by the flotation method.

Table 2: Comparative table of parasites obtained using sedimentation and flotation methods

Methods / Turtles N°	Sedimentation	Flotation
1	6	5
2	5	1
3	2	2
4	2	1
5	4	0
6	8	0
7	8	2

8	6	1
9	7	3
10	8	2
11	6	3
12	6	2
13	6	5
14	6	2
15	5	2
16	4	1
17	7	0
18	6	0
19	5	1
20	4	1
21	1	1

III.5. Correlation of parasites with sex, weight and age of tortoises

a- Sex

The distribution of parasites according to turtle sex is shown in Table 3.

Table 3: Contingency table of turtle sex and parasites

Number de parasites / Sexe	2	3	4	5	6	7	8	10
Femeale	0	0	1	0	0	1	1	1
Male	1	2	2	1	5	3	3	0

- Age

According to Spearman's correlation test between the distribution of parasites and the age of the tortoise, there is a weak correlation between these two variables with a value of 0.4072. The figure below shows the scatterplots of the correlation between these two variables (Figure 4).

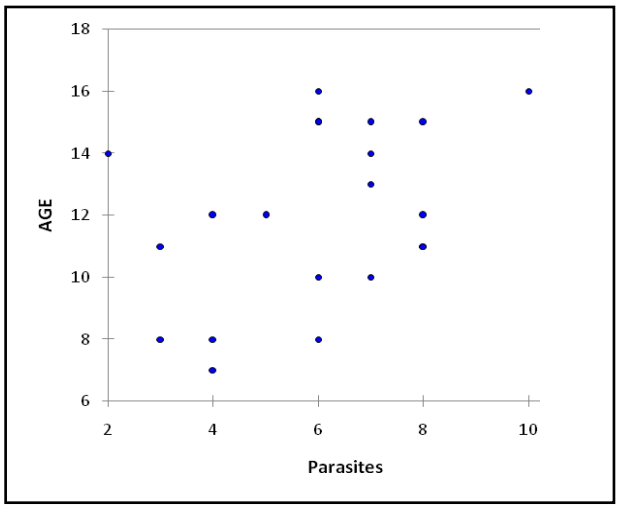

Figure 4: Correlation between parasites and turtle age

Weight

After the Spearman test, a weak correlation exists between parasites and turtle weight, with a correlation coefficient of 0.2025. The scatterplots (Figure 6) below represent the point levels for each turtle.

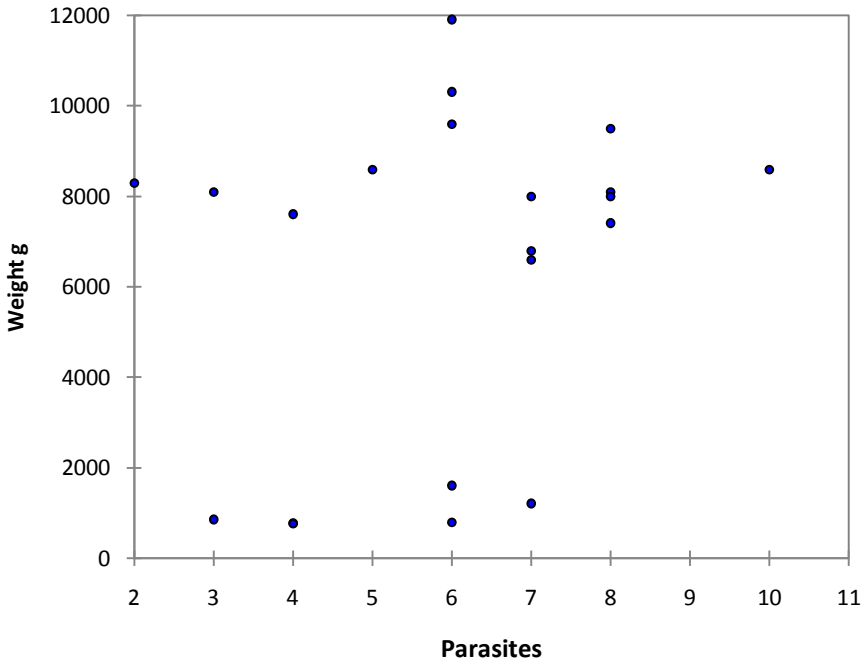

Figure 6: Scatter plot of parasite distribution in relation to turtle weight

IV. Conclusion

Parasites can have harmful effects on the health of host organisms. Their effects are not instantaneous, but manifest themselves slowly on the organisms and finally pass to the engraved state if there is no treatment. This

study provided a better understanding of the helminth populations of Astrochelys radiata conserved in the Tsimbazaza Botanical and Zoological Park, in order to gain knowledge of the parasite rate in this host.

The study not only identified the parasites detected in the Park's radiata turtles, but also determined the rate of parasite infestation in each radiata turtle. Twenty species of parasite were detected during the study. These identified species fall into 3 classes: Nematodes, Sporozoa and Litostomatea. As regards the rate of infestation, the parasites are known in all the tortoises which were made the sampling of their faeces. In the counting that we had observed that at least 50 parasites were detected in the 1 g of feces. Parasite infestations in turtles correlate with age, with adult turtles containing higher numbers of parasites than young turtles. On the other hand, parasites were also positively correlated with turtle weight. Parasites are therefore found in hosts that can meet their needs.

This work could add to the study of parasites in the chelonians of Madagascar, where little research has been carried out, as researchers have focused more on other animals such as carnivores, which are not concerned with reptile groups. The treatments described in this study will enable the reader to improve turtle health and combat turtle parasites.

However, this study is far from complete, which prompts us to formulate other recommendations.

Research prospects to fill the gaps identified.

There are several possible reasons for the parasites affecting *Astrochelys radiata* turtles in the Park.

A study in parasite taxonomy should be useful in order to identify parasites not identified in publications by researchers. In this way, errors in parasite identification can be avoided.

Research into effective treatments and remedies for parasites should improve the health of turtles.

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