Haemogregarine Genetic Diversity in Captive African Rock Pythons from Nigeria Suggests a Geographical Pattern

Nijerya'daki Afrika Kaya Pitonlarındaki Hemogregarin Genetik Çeşitlilik Coğrafik Bir Paterni Göstermektedir

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ABSTRACT

Objective: Understanding the processes that drive parasite diversification, distribution, and abundance is central to disentangle the dynamics and evolution of diseases. In this study, we screened African rock pythons from Nigeria for the presence of blood parasites to assess their distribution, diversity, and phylogenetic relationships.
Methods: A total of 21 captive African rock pythons collected from across 11 locations in Nigeria were sampled between August 2016 and January 2017. Samples were microscopically and genetically analyzed.
Results: From the blood smears analyzed, 10 (47.6%) snakes were found to be infected with haemogregarines. Eight of the infected samples were genetically assessed and confirmed as haemogregarines of the recently described *Bartazoon* group. Two haplotypes were retrieved, of which one was distributed in the northern-central sampled localities and the other in the southern localities. The two haplotypes were clustered in a clade of haemogregarines from snake, gecko, and rodent hosts, and among them, the haemogregarine species *Hepatozoon ayorgbor* was described from the ball python *Python regius.*
Conclusions: Two haemogregarine haplotypes in Nigerian rock pythons, which appear to have a geographical pattern across the country, were detected in this study.

Keywords: Hemoparasites, apicomplexa, haemogregarines, snake, African rock python, phylogenetic analysis, Africa
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ÖZ

Amaç: Parazit diversifikasyonu, dağılımı ve bolluğunu yönlendiren süreçlerin anlaşılması hastalıkların dinamikleri ve evrimlerini çözmek için önemlidir. Bu çalışmada Nijerya'daki Afrika kaya pitonları dağılım, çeşitlilik ve filogenetik ilişkilerini değerlendirmek amacıyla kan parazitlerinin varlığı açısından incelendiler.
Yöntemler: Ağustos 2016 ile Ocak 2017 arasında Nijerya'daki 11 farklı bölgeden alınan 21 Afrika kaya pitonu örneklendirildiler. Örnekler mikroskopik ve genetik olarak analiz edildiler.
Bulgular: Kan yayma analizlerinde 10 pitonun (%47,6) hemogregarinler tarafından enfekte oldukları bulundu. Enfekte numunelerin sekizi genetik açıdan değerlendirildi ve son zamanlarda tanımlanan *Bartazoon* grubunun hemogregarinleri olarak konfirme edildiler. İki haplotip yeniden kazanıldı. Bunlardan birisi kuzey-merkez bölgelerde ve diğeri de güney bölgelerde dağılım gösterdi. İki haplotip yılan, geko ve kemirgen konaklardan alınan bir hemogregarin türünde kümelendiler. Bunlardan hemogregarin cinsi *Hepatozoon ayorgbor* top pitondan (*Python regius*) tanımlandı.
Sonuç: Bu çalışmada Nijerya kaya pitonlarında, tüm ülkede coğrafik bir paterninin olduğu görülen iki hemogregarin haplotipi tespit edildi.

Anahtar sözcükler: Hemoparazitler, apicomplexa, hemogregarinler, yılan, Afrika kaya pitonu, filogenetik analiz, Afrika
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INTRODUCTION

The phylum Apicomplexa is notable for its high medical and veterinary importance, particularly owing to the impacts of *Plasmodium*, *Babesia*, and *Toxoplasma* among others (1). Regardless of this, the knowledge on the taxonomical diversity of this phylum remains relatively limited. It has been estimated that only 0.1% of the Apicomplexan species have been described (2). Moreover, within this percentage, there is a bias in studies toward a few genera, being even less known regarding the parasites of wild hosts, including their identity, evolutionary relations, and ecology (3). Having this basic information could greatly improve the conservation efforts of hosts as well as increase the overall knowledge on Apicomplexa. Haemogregarines are geographically ubiquitous parasites that infect all groups of terrestrial vertebrates and an array of hematophagous arthropods (4). They present various life cycles, which usually involve an invertebrate vector as the definitive host and a vertebrate as the intermediate host but might also include other vertebrates as paratenic hosts upon whom the intermediate hosts prey. The use of prey–predator networks by these parasites has been shown by experimental transmission studies (5, 6) and supported by phylogenetic assessments (7). However, other factors, such as vertebrate host distribution, habits, and relatedness and their exposition to vectors have also been suggested to play a role in shaping the presence, frequency, and diversity of haemogregarines in host communities (7-9). Recent phylogenetic studies have also identified taxonomical incongruences, suggesting that the taxonomy of terrestrial haemogregarines should be rearranged and divided into four genera: *Hepatozoon*, *Hemolivia*, *Karyolysus*, and the newly created *Bartazoon* (10). Members from all these four genera have been found infecting reptiles; haemogregarines are one of the most commonly reported blood parasites, particularly in reptiles (1). Although haemogregarines are generally considered apathogenic in reptiles, the degree of the effect differs among studies, from no apparent effect to severe health effects (11). In this study, we screened African rock pythons from Nigeria for the presence of these parasites to assess their distribution, diversity, and phylogenetic relationships.

METHODS

Sampling

A total of 21 captive African rock pythons from 11 locations in the northern, central, and south-western parts of Nigeria across eight states were sampled between August 2016 and January 2017 (Figure 1a for the sampling map). Each snake was subjected to physical and clinical examinations, sexed, weighed, and measured. Blood was collected from the ventral coccygeal vein, as described by Lock (12), and stored in heparinized tubes for later use. Blood smears were examined for parasite detection using a light microscope. In ophidian hosts, haemogregarines are easily detected by observing their stages inside red blood cells (1). Photomicrographs were taken using a Magnus Fixed microscope adapter (FMA050 3MP) attached to a computer (MagnusPro 3.7 software). Blood from infected pythons was transferred to Whatman Flinders Technology Associates cards for molecular identification.

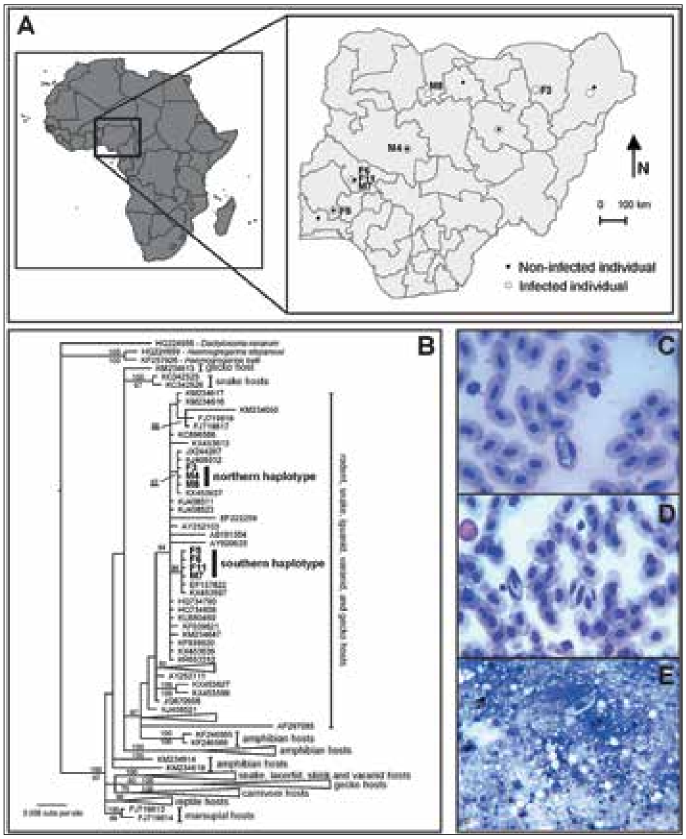

Figure 1. a-e. Sampling locations, phylogenetic tree of detected haemogregarine, and selected photographs of the parasites. (a) Map of the study area containing locations of the infected (white dots) and non-infected (black dots) individuals. Some location points overlap. (b) Estimates of the evolutionary relationships (pictured is the tree derived from the Bayesian phylogenetic analysis). Bayesian posterior probabilities are presented above nodes and bootstrap values for the maximum likelihood analyses are presented below them (only values >75 for the former and 70 for the latter are shown). (c) Haemogregarine photograph 1. (d) Haemogregarine photograph 2. (e) Haemogregarine gametocyte and possible sporocysts (black arrow) in the hemocoel of an Aponomma tick attached to one of the sampled snakes

DNA Extraction and Amplification

DNA was extracted using standard high-salt methods (13). Polymerase chain reaction (PCR) was performed using primers specific for a 600-bp long region of the 18S rRNA gene, HepF300, and HepR900 (14). PCR was performed as described by Harris (15). Amplified products were purified and thereafter sequenced by an external company (Beckman Coulter Genomics, UK). Sequences were blasted in the GenBank database to confirm the identity of the amplified products. Sequences were corrected and aligned in Geneious v5.6.7, Biomatters Limited, New Zealand (16) using the MUSCLE algorithm (17). For phylogenetic analyses, GenBank sequences of other haemogregarines were added to the dataset, which resulted in a final dataset that included 145 sequences and was 584-bp long. The substitution model of evolution was chosen according to the Bayesian information criterion selected by jModelTest 2 (model 012030+G+F) (18). Phylogenetic relationships were estimated using the maximum likelihood (performed in

PhyML 3.1, South of France bioinformatics platform, Montpellier, France) (19) and Bayesian inference (Mr. Bayes v.3.2.6 developed by Ronquist et al. (20) methods) (Figure 1b). For more details on parameters, see Tomé (21).

RESULTS

All pythons were apparently healthy at the time of blood collection based on physical examination. From the 21 blood smears analyzed under the microscope, 10 (47.6%) snakes were found to be infected, with haemogregarines being the suspected parasite in all cases (Figure 1c and d). Eight of the infected samples were genetically assessed; however, all attempts of PCR amplification failed in one of the samples. All the seven obtained sequences belong to the *Bartazoon* haemogregarine group, as identified by Karadjian (10), in a well-supported clade of haemogregarines from snake, gecko, and rodent hosts, but in two distinct lineages (Figure 1b). The haemogregarines infecting pythons from the southwestern region of Nigeria grouped with *Hepatozoon ayorgbor*, described from the ball python *Python regius* in Ghana, and a sequence retrieved from *Hemidactylus alkiyumii* and the Oman saw-scaled viper *Echis omanensis*, both from Oman. The remaining four positive samples were from pythons of the north and central parts of Nigeria. They were grouped with sequences from a white-lipped Herald snake *Crotaphopeltis hotamboeia* from Niger, a horseshoe whip snake *Hemorrhois hippocrepis* from Spain, the Arabian horned viper *Cerastes gasperettii*, and the diademed sand snake *Lytorhynchus diadema* from Oman.

DISCUSSION

Haemogregarines have been previously reported in two python species (14, 22-25) and other snakes from Africa (9, 24, 26, 27). For snakes of tropical Africa, prevalence appears to increase in captivity, with values of 47.6% (in the present study) and 78.2% in imported snakes from Ghana (22). In contrast, wild snakes were less commonly infected, presenting frequencies of approximately 14% (24, 26) and 16.7% (25). There is not yet a consensus on the effect of haemogregarines on their reptile hosts. While some authors have reported none or little effect, higher parasitemia levels have been reported to be associated with erythrocyte hemolysis, anemia, and mortality (11, 28-32). However, the patterns observed appear to suggest that captivity increases the predisposition to infection, and such information should be considered in captivity and conservation programs.

Several haemogregarine species have been described from python hosts [Smith (4) included a list of available species description from the bibliography, and more recently, Sloboda et al. (22) described *H. ayorgbor*]. Unfortunately, molecular data are only available for *H. ayorgbor*; therefore, it is not possible to determine whether the haemogregarine haplotypes detected here belong to any described species. However, because the haplotype infecting southern Nigerian rock pythons shares the same 18S rRNA sequence as that of *H. ayorgbor*, it can be suspected that it belongs to this species or at least to a very closely related species. Other haemogregarine haplotypes infecting pythons have also been reported. Rosado et al. (25) screened the samples of *Python sebae* from Mauritania, Senegal, and Mali and detected a distinct haemogregarine haplotype and Haklová-Kočíková et al. (24) sampled pythons (*Python natalensis* and *P. regius*) from Swaziland and found one to be infected. Unfortunately, the region of the 18S rRNA analyzed in this study did not overlap with the sequence retrieved by Haklová-Kočíková et al. (24), and, thus, it was not included in the present phylogenetic analysis. However, Haklova's haplotype belongs to the same clade of haemogregarine as that in our study and that of others identified from pythons. Thus, based on the information available to date, African pythons, specifically rock pythons, are infected by three (possibly four) haemogregarine haplotypes.

A striking pattern in our results is the geographical division in the distribution of the two detected haplotypes, with one haplotype occupying the northern and central parts of Nigeria and the other occupying the southern part. Notably, Nigeria presents a latitudinal climatic variation, with a dryer savannah north and a tropical south (33), which might correlate with the distribution pattern of the haemogregarines. This is also supported by the grouping of the northern haplotype with haemogregarines from snakes of Niger and Spain (both countries with dryer climates) and those of the southern haplotype with a haemogregarine species described from Ghana (a tropical country). In contrast, sequences of haemogregarines from snakes in Oman (9) also grouped with both haplotypes. This might happen for several reasons but contradicts the climatic explanation for the pattern (or at least as the only explanation). In this study, we did not seek to identify the definitive host of these haemogregarines. However, the tick *Aponomma latum* was found infecting some snakes, and a gamont and suspected sporocyst was observed in a slide preparation from one of these ectoparasites (but no developmental stages in the hemocoel) (Figure 1e). Conversely, Sloboda et al. (22) established the role of the mosquito *Culex quinquefasciatus* as a vector in the experimental transmission of *H. ayorgbor*. Because the haplotypes detected are closely related to this species, they might have the same type of definitive host. Only further experiments can confirm this, but additionally, the identity of the definitive host might offer clues to explain the geographical pattern in the distribution of these haemogregarines, along with other factors such as differences in diet and genetic structuration of the python hosts.

CONCLUSION

To our knowledge, this is the first study on the presence and molecular assessment of blood parasites infecting snakes from Nigeria. Two haemogregarine haplotypes infecting Nigerian rock pythons genetically identified as part of the *Bartazoon* group, which appear to be differently distributed across the country, were detected in this study. Further studies are required to identify the possible definitive hosts of the haemogregarine parasites and to expand the sampling to other hosts and localities to assess whether the uncovered geographical pattern is due to climatic differences, particular of the host group, or distorted by the captivity.

Ethics Committee Approval: Ethics committee approval was received for this study from the University of Ibadan Animal Care and Use Research Committee.

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