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Non-hemoparasitic protozoa of the subdesert toad, *Amietophrynus (Bufo) xeros* (Anura: Bufonidae)

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Abstract
Eighty subdesert toads, *Amietophrynus (Bufo) xeros,* were collected in Shendi, Sudan, between August and December 2014 and examined for the presence of non-hemoparasitic protozoa. Fifty-four (67.5%) of the toads were found to be infected with one or more protozoa including, *Nyctotherus* sp., *Balantidium* sp. *Opalina* sp. and *Protoopalina* sp. A higher prevalence of infection was found in female toads, while a higher intensity of infection was found in males. In addition, older toads harbored a higher prevalence and intensity of infection. No significant correlations were found between the gender of the toads examined and either the prevalence or intensity of infection of the protozoan species. Likewise, the prevalence and intensity of infection by protozoan species were not significantly correlated with the snout-vent length of the toads.

Keywords: *Amietophrynus (Bufo) xeros*, Protozoa, Prevalence, Intensity, Sudan.

1. Introduction
The subdesert toad, *Amietophrynus (Bufo) xeros* is a medium-sized terrestrial Anura, which lives in arid regions of Africa close to permanent water bodies, dry riverbeds and around oases [1, 2]. This species has been classified as being of least concern, according to the International Union for the Conservation of Nature Red List criteria [3]. Within Sudan, *A. xeros* is found in the northern parts where it inhabits local moist farms and gardens. This species is mainly nocturnal and feeds on small invertebrates.
It is well known that amphibians have a rich parasite fauna including viruses, bacteria, fungi [4, 5], protozoa [6, 7], helminthes [8, 9] and mites [10]. However, to date, no studies exist dealing with the protozoan parasites of the subdesert toad, *A. xeros*. Therefore, the purpose of the present study was to report the protozoan fauna of this toad in Shendi, a city located in the northern part of Sudan and to establish the prevalence and intensity of protozoan species infection in relation to the age and gender of the toad.

2. Materials and Methods
Eighty *A. xeros* toads were collected by hand from around water ponds in the agricultural lands in Shendi (16°40'N, 33°25'E), Sudan, from August to December 2014.
The specimens were immediately transferred to a laboratory and sacrificed using chloroform, then their SVL was measured with calipers to the nearest 0.1mm. In the necropsy, the sex of each toad was recorded by direct observation of the gonads, then the internal organs: esophagus, stomach, small intestine, large intestine and urinary bladder were removed, dissected and placed in separate Petri-dishes containing 10mL of normal saline solution 0.9%. The protozoa were collected by pipetting them from the organs' content and examining them under a light microscope either alive or from smears stained with methylene blue 0.1% or Lugol's iodine solutions. Quantification of the protozoa was conducted by smearing 1mL of the stained homogenized content of each organ onto a slide and thereafter, observing it thoroughly at 100-400x magnification. The number of protozoan species found in 1mL from each host organ was then multiplied by ten to estimate the total number of protozoa present. Identification of the protozoa was conducted using the guidelines by Kudo [11]. The toads were grouped into two age classes (juvenile: SVL < 40mm, and adult: SVL ≥ 40mm) based on the observation that individuals with a SVL below 40mm did not have differentiated gonads.
The prevalence and intensity of infection were calculated in accordance with the method used by Bush [12]. The relationship of the prevalence and intensity of infection to host gender and SVL (age) were calculated using the Pearson correlation coefficients (r). The statistical

software, SPSS 16.0 for Windows was used for the data analysis and values were considered significant when $P < 0.05$.

3. Results

Out of the 80 *A. xeros* toads, 54 (67.5%) were found to be infected with one or more internal protozoa, including *Nyctotherus* sp., *Balantidium* sp., *Opalina* sp. and *Protoopalina* sp. All of these protozoa were recovered from the large intestines of the specimens examined. Of the 80 specimens, 25 (31.3%) harbored one species, 23 (28.8%) harbored two species, 5 (6.3%) harbored three species, and one (1.3%) harbored four species. The prevalence, mean intensity and intensity range of infection of the protozoans detected are presented in Table 1.

Table 1: Prevalence, mean intensity and intensity range of infection by protozoan species in *A. xeros* toad (n = 80).

Protozoan species	Prevalence %	Mean intensity	Intensity range
Nyctotherus sp.	45.00	431.4	50-2000
Balantidium sp.	10.00	587.5	100-200
Opalina sp.	36.20	458.3	80-1700
Protoopalina sp.	21.20	386.5	50-1800

A relatively high prevalence of infection was observed among female toads compared with males (Fig. 1), although this difference was not found to be statistically significant in a paired sample *t* test ($P > 0.05$). In addition, a higher prevalence was observed among older toads (SVL 40-80mm) when compared with younger ones (SVL<40mm), (Fig. 2), and this difference was statistically significant ($P < 0.05$). A higher intensity of infection was observed among male toads compared with females (Fig. 3), but, the difference was not found to be statistically significant ($P > 0.05$). Likewise, older toads harbored a higher intensity of infection than younger ones (Fig. 4), but again, the difference was not statistically significant ($P > 0.05$).

There was no significant correlation between the gender of the toads examined and either the, prevalence or intensity of infection of any of the protozoan species ($r = -0.001$ to 0.19, $P > 0.05$). In addition, the prevalence and intensity of protozoan species were not significantly correlated with the SVL and hence the age of the toads ($r = -0.02$ to 0.19, $P > 0.05$).

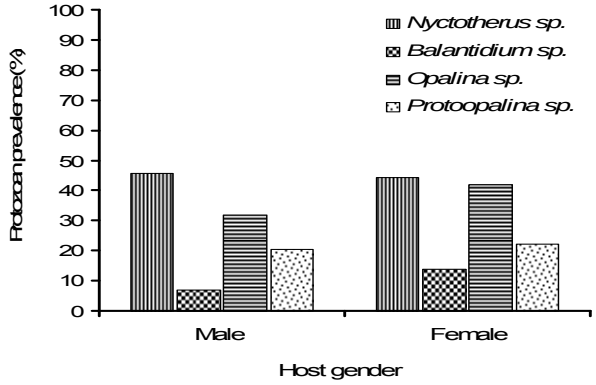

Fig 1: Prevalence of protozoan infection among *A. xeros* toads, according to gender, (male: n = 44; female: n = 36).

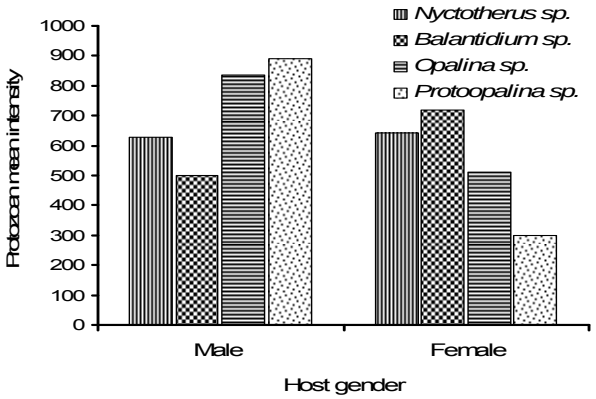

Fig 2: Mean intensity of protozoan infection among *A. xeros* toads, according to gender, (male: n = 44; female: n = 36).

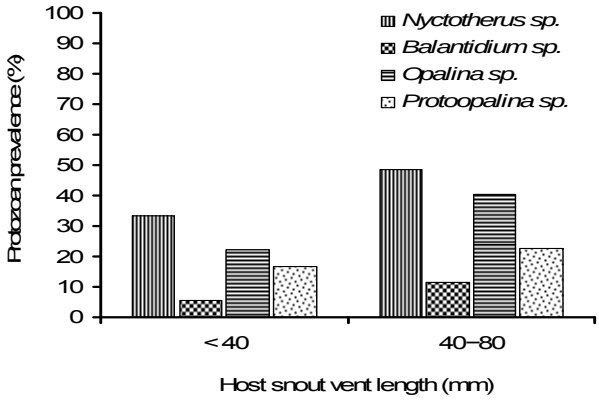

Fig 3: Prevalence of protozoan infection among *A. xeros* toads, according to SVL, (< 40mm: n = 18; 40-80mm: n = 62).

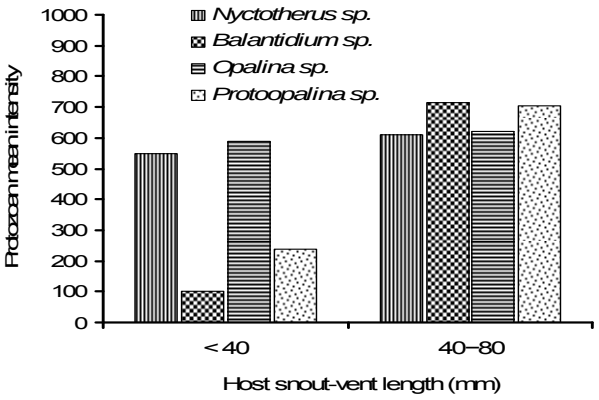

Fig. 4. Mean intensity of protozoan infection among *A. xeros* toads, according to SVL, (< 40mm: n = 18; 40-80mm: n = 62).

4. Discussion

In the present study, the subdesert toad, *A. xeros* was found to be parasitized by different protozoa including, *Nyctotherus* sp., *Balantidium* sp., *Opalina* sp. and *Protoopalina* sp. The protozoa identified have previously been reported in amphibian species [13, 14]. The ciliate protozoa of the genus *Nyctotherus,* are cosmopolitan in their distribution and have been described from different animals including reptiles, amphibians and insects [14, 15]. Likewise, the genus *Balantidium* has a cosmopolitan distribution and more than 18 species been reported from various amphibians [13, 16]. The opalind protozoa of the genera, *Opalina* and *Protoopalina* are all

endocommensals and mainly inhabit the large intestines of various amphibians [17, 18] including frogs such as *Hyla chrysoscelis* [19], *Xenopus laevis* [20] and *Pseudacris fouquettei* [14].

In the present study, a relatively high prevalence of infection was observed among female and old aged toads although previous studies carried out on anurans have found no relationship between the gender of the host and the parasite community structure [9, 21]. Differences in the parasite prevalence between female and male toads are probably due to ecological or behavioral differences between the sexes. On the other hand, some studies have documented a decrease in the prevalence of parasite infections based on host age [22, 23], while others have reported no relationship with age [24, 25].

In the present study, no significant correlation was found between the gender or age of the toads examined and either the prevalence or intensity of infection. However, it has previously been reported that the prevalence of parasite infection is correlated with the SVL of the host [26, 27]. An increase in the prevalence or intensity of infection with age may be caused by prolonged exposure to parasite accumulation or the existence of a larger space for the parasites to feed and develop [28]. The protozoa found in this study live commensally in the intestine of various amphibians with direct life cycles and this may result in the high frequency of infection in the host specimens. Moreover, the locality in which the host specimen lives is one of the most important ecological factors which plays a substantial role in the occurrence of parasite species and hence, host infection.

5. Conclusion

This is the first study to report on the non-hemoparasitic protozoa that infect the subdesert toad, *A. xeros* in Shendi area, Sudan. Thus, further studies are required, in order to classify these protozoa down to their species level.

6. Acknowledgments

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