ISSN- 2350-0530(O) ISSN- 2394-3629(P)
Impact Factor: 2.035 (I2OR)
DOI: https://doi.org/10.29121/granthaalayah.v3.i10.2015.2934

INTERNATIONAL JOURNAL OF RESEARCH – GRANTHAALAYAH
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Science

HELMINTH PARASITES OF THE SUBDESERT TOAD, AMIETOPHRYNUS (BUFO) XEROS (ANURA: BUFONIDAE)

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ABSTRACT

Sixty-nine subdesert toads, Amietophrynus (Bufo) xeros, were collected in Shendi, Sudan, from August to November 2014, and examined for the first time for the presence of internal helminth parasites. Sixty-seven (97.1%) of the toads were found infected with one or more helminth species including, four Nematoda: Aplectana macintoshii, Rhabdias bufonis, Strongyloides prokopici, and Oswaldocruzia sp.; one Trematoda: Mesocoelium sp.; one Cestoda: Lanfrediella sp. A higher prevalence and intensity of infection was found in male toads compared with females, and old toads were found to harbor a higher prevalence and intensity of infection when compared to younger ones. However, neither of these differences was statistically significant. In addition, there were no significant correlations found between the gender of the toads examined and either the prevalence or intensity of infection of any of the helminth species. Likewise, the prevalence and intensity of infection of the helminth species were not significantly correlated with the snout-vent length of the toads.

Keywords:
Amietophrynus (Bufo) xeros; Helminth parasites; Prevalence; Intensity; Sudan.

Cite This Article: Yassir Sulieman, Azzam Afifi, Higazi M. Awad, and Theerakamol Pengsakul, "HELMINTH PARASITES OF THE SUBDESERT TOAD, AMIETOPHRYNUS (BUFO) XEROS (ANURA: BUFONIDAE)" International Journal of Research – Granthaalayah, Vol. 3, No. 10(2015): 75-83. DOI: https://doi.org/10.29121/granthaalayah.v3.i10.2015.2934.

1. INTRODUCTION

The subdesert toad, Amietophrynus (Bufo) xeros is a terrestrial Anura, lives in arid regions of Africa close to permanent water bodies, dry riverbeds and around oases (Rödel, 2000; Harper et al., 2010). This species has been classified as least concern, according to the International Union for the Conservation of Nature Red List criteria (IUCN, 2013). Within Sudan, A. xeros is found in

the northern parts; inhabits local farms, gardens and orchards, and is well adapted to anthropogenic areas. It is mainly nocturnal and feeds on small invertebrates such as spiders, beetles and other insects. It is well known that amphibians have a rich parasite fauna, including viruses, protozoans and helminths (Canning et al., 1964; Hyatt et al., 2000; Duszynski et al., 2007; Rahman et al., 2008; Sulieman and Pengsakul, 2015). Several studies have been carried out on the protozoan and helminth parasites of Amphibia (Mcallister et al., 1995; Dare et al., 2008; Santos et al., 2013). However, to date, no studies exist on the parasite fauna of A. xeros. Therefore, the present study will provide a baseline of information on the following: (i) the helminth parasites of this toad species in Shendi, a city located in the northern part of Sudan (ii) the prevalence and intensity of helminth infections in relative to the toads' age and gender.

2. MATERIALS AND METHODS

From August to November 2014, 69 *A. xeros* toads were collected manually from the agricultural lands and orchards in Shendi (16°40'N, 33°25'E), Sudan. The specimens were immediately transferred to the Laboratory of Zoology, University of Shendi, and sacrificed using chloroform. The snout-vent length (SVL) and gender were recorded for each individual. The hosts were grouped into two age classes on the basis of their SVL (juveniles: < 40 mm, and adults: 40-80 mm) based on the observation that individuals with a SVL below 40mm did not have differentiated gonads. In the necropsy, the internal organs: lungs, heart, liver, esophagus, stomach, small intestine, large intestine, urinary bladder and kidneys were removed, dissected and placed in separate petri-dishes containing 0.9% normal saline solution and examined thoroughly under a stereomicroscope for helminth parasite infections. The helminth isolated were prepared following Leon-Regagnon et al. (2005), then identified under a compound microscope based on the works of Baker and Vaucher (1986), Melo et al. (2011) and Calhoun and Dronen (2012). The prevalence and intensity of infections were calculated in accordance with Bush et al. (1997). The SPSS 16.0 analytical software was used for the statistical analysis. The relationship of the prevalence and intensity of infection to host gender and SVL were calculated using Pearson correlation coefficients (r) and paired sample tests were used to compare the intensity and prevalence of infection based on the specimens' gender and age (according to SVL). The helminth specimens were preserved in 70% ethanol and deposited in the Department of Zoology, Faculty of Science and Technology, University of Shendi.

3. RESULTS

Out of the 69 *A. xeros* collected, 67 (97.1%) specimens were found infected with one or more internal helminth parasite species of either nematode or platyhelminth or both. Six different helminth species were recovered from the internal organs of the toads examined including, Nematoda: *Aplectana macintoshii*, *Rhabdias bufonis*, *Strongyloides prokopici*, and *Oswaldocruzia* sp.; Trematoda: *Mesocoelium* sp.; Cestoda: *Lanfrediella* sp. The prevalence, mean intensity and site of infection of these helminths identified were given in table1. Of the 69 specimens examined, 29 (42.03%) harbored one species, 25 (36.23%) harbored two species, 12 (17.40%) harbored three species, and one (1.45%) harbored four species.

There was no significant correlation found between the gender of the toads examined and either the, prevalence or intensity of infection of any of the helminth species ($r = -0.03$ to 0.13, $P = 0.17$

to 0.98). Likewise, the prevalence and intensity of helminth species were not significantly correlated with the SVL of the toad species (r = − 0.01 to 0.20, P = 0.08 to 0.96).

A relatively high prevalence of infections was observed among male toads compared with females (Fig. 1), although this difference was not statistically significant (P = 0.86). Likewise, a high prevalence was observed among old toads (SVL 40-80 mm) when compared with younger ones (SVL < 40 mm), (Fig. 2), but this difference was not statistically significant (P = 0.12). Similarly, a high intensity of infections was observed among male toads compared with females (Fig. 3), however, the difference was not statistically significant (P = 0.27). Finally, old toads harbored a higher intensity of infections than younger ones (Fig. 4), but again, the difference was not statistically significant (P = 0.59).

4. DISCUSSION

In the present study, six helminth taxa were recorded from the subdesert toad, *A. xeros* including, Nematoda: *Aplectana macintoshii*, *Rhabdias bufonis*, *Strongyloides prokopici* and *Oswaldocruzia* sp.; Trematoda: *Mesocoelium* sp.; Cestoda: *Lanfrediella* sp. All of the helminths identified have previously been reported to parasitize several anuran species (Baker, 1987; Kuzmin et al., 2007; Dronen et al., 2012). However, no previous studies exist regarding the parasite fauna of the toad, *A. xeros*. The nematode, *Aplectana macintoshii* was the most common parasite found to be harbored by *A. xeros* in this study. This species belongs to the family Cosmocercidae, which includes approximately 41 species and the majority of which parasitize the intestine of frogs and toads; those species are found in North America, Europe, Asia, South America and Africa (Baker, 1980; Adamson and Baccam, 1988). The genus *Strongyloides* is usually an intestinal parasite of vertebrates, but has previously been reported as infecting amphibians (Moravec et al., 1987; Patterson-Kane et al., 2001). Likewise, the genus *0swaldocruzia*, family Molineidae is a cosmopolitan parasite of amphibians and reptiles (Ben Slimane and Durette-Desset, 1996). The genus *Rhabdias*, family Rhabdiasidae is a parasite in the lungs of amphibians and reptiles (Baker, 1987). It is cosmopolitan in distribution and lives in any environment that supports its host frog and toad species (Goater, 1992). The cestode *Lanfrediella*, is a new genus belonging to the family Nematotaeniidae, and it has been reported from the intestine of the toad *Rhinella marina* (Melo et al., 2011). The trematode genus, *Mesocoelium*, belongs to the family Mesocoeliidae, which contains a total of 49 species widely parasitizing amphibians, reptiles and fish (Dronen et al., 2012).

In the present study, the main group of helminths found parasitizing *A. xeros* were nematode species; this result is consistent with several previous findings on the toads, *Bufo ictericus* (Luque et al., 2005), *Amietophrynus regularis* (Ibrahim, 2008) and *Rhinella icterica* (Santos et al., 2013). This is because, on land, anurans are more exposed to nematodes with monoxenous life cycles such as *Strongyloides* spp., *Rhabdias* spp., and cosmocercids, because the majority of nematodes infect anurans through cutaneous penetration or the ingestion of eggs. Similarly, previous studies have shown that the bufonid amphibians tend to harbor a higher number of nematode species than trematodes, as was seen in this study (Bolek and Coggins, 2003), this is likely to be because the terrestrial toads predominantly feed on ants, beetles and other terrestrial invertebrates (Hirai and Matsui, 2002) and are therefore less prone to infection from the wide range of species of trematodes which commonly infect aquatic amphibians. Additionally, it has been reported that

there is a greater incidence of infection of anurans with nematodes than with cestodes infections (Mohammad, 2010).

A high prevalence and intensity of infection was observed among old male toads in the present study. *However,* previous studies carried out on anurans found no relationship between the gender of the host and the parasite community structure (Santos and Amato, 2010; Santos et al., 2013). Some studies have documented a decrease in the prevalence of parasite infections according to host age (Ibrahim, 2008; Tinsley et al., 2012), while others have reported no relationship with age (Garvin et al., 2003; Hasselquist et al., 2007). No significant correlations were found between gender and either the prevalence or intensity of infection with any helminth species in this study. Likewise, the prevalence and intensity of helminth infection were not significantly correlated with the SVL of the host toads. However, it has previously been reported that the prevalence of parasitic infections is correlated with the SVL of the water frog *Pelophylax* kl. *Hispanicus* (Comas et al., 2014), and there have also been some other same findings of host/parasite relationships (Haas et al., 2012; Treml et al., 2012). An increase in prevalence with age may be caused by prolonged exposure to parasite accumulation (Sanchis et al., 2000). However, some nematode populations diminish in larger hosts, because they become more resistant to new reinfections (Baker, 1979), and have thicker skins protecting them from parasite penetration (Barton, 1998). In addition, other ecological factors may have an influence on the parasite composition of the host.

Finally, this is the first study to establish a list of the helminth parasites that infect the subdesert toad, *A. xeros* in Sudan. Thus, further studies are required, including studies of their taxonomic aspects.

5. ACKNOWLEDGEMENTS

The authors would like to thank Hassan Awad and Abbas Hassan, University of Shendi for their help in the collection of samples.

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ISSN- 2350-0530(O) ISSN- 2394-3629(P)
Impact Factor: 2.035 (I2OR)

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Table 1: Prevalence, mean intensity (range), and site of infection of helminths in *A. xeros* toad (n = 69), from Shendi, Sudan.

Helminth species	Prevalence (%)	Mean intensity (range)	Site of infection
Nematoda			
Aplectana macintoshii	91.30	20.21 (2-55)	Large intestine
Rhabdias bufonis	17.40	17.33 (3-80)	Lungs
Strongyloides prokopici	13.04	2.44 (1-5)	Small intestine
Oswaldocruzia sp.	11.60	3.40 (2-5)	Small intestine
Trematoda			
Mesocoelium sp.	18.84	44.4 (2-150)	Small intestine
Cestoda			
Lanfrediella sp.	20.30	3.30 (1-8)	Small intestine

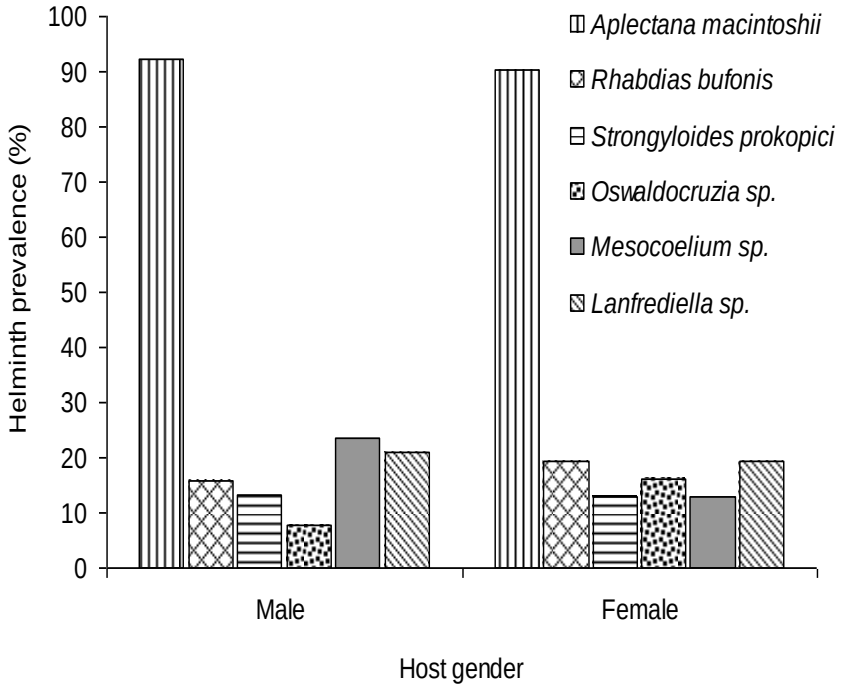

Figure 1: Prevalence of helminth infections among *A. xeros* toads, *according to gender, (male: n = 38; female: n = 31).*

ISSN- 2350-0530(O) ISSN- 2394-3629(P)
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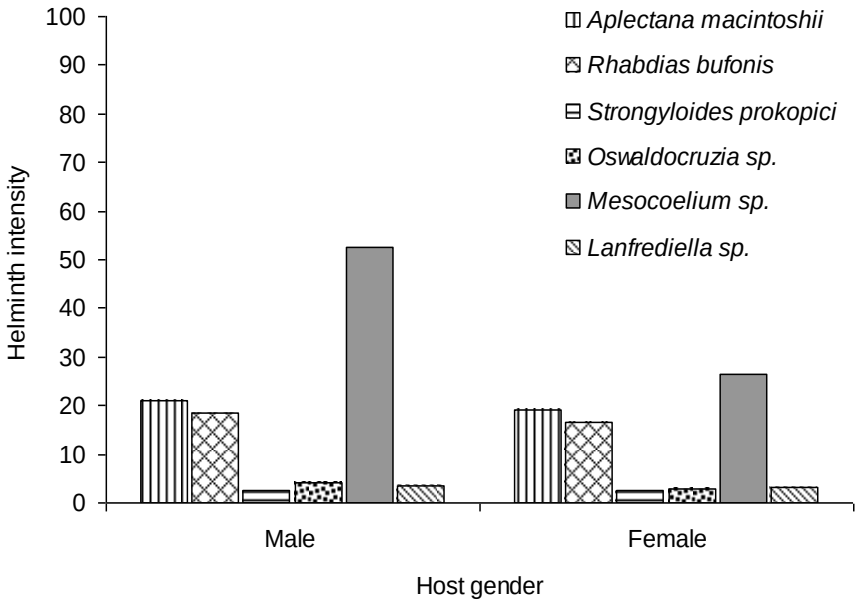

Figure 2: Intensity of helminth infections among *A. xeros* toads, *according to gender, (male: n = 38; female: n = 31).*

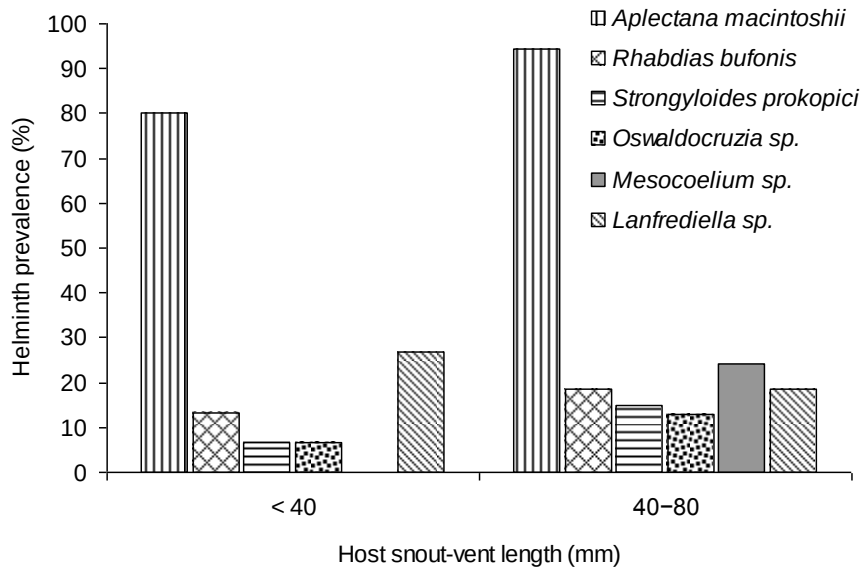

Figure 3: Prevalence of helminth infections among *A. xeros* toads, *according to SVL, (< 40 mm: n = 15; 40-80 mm: n = 54).*

ISSN- 2350-0530(O) ISSN- 2394-3629(P)
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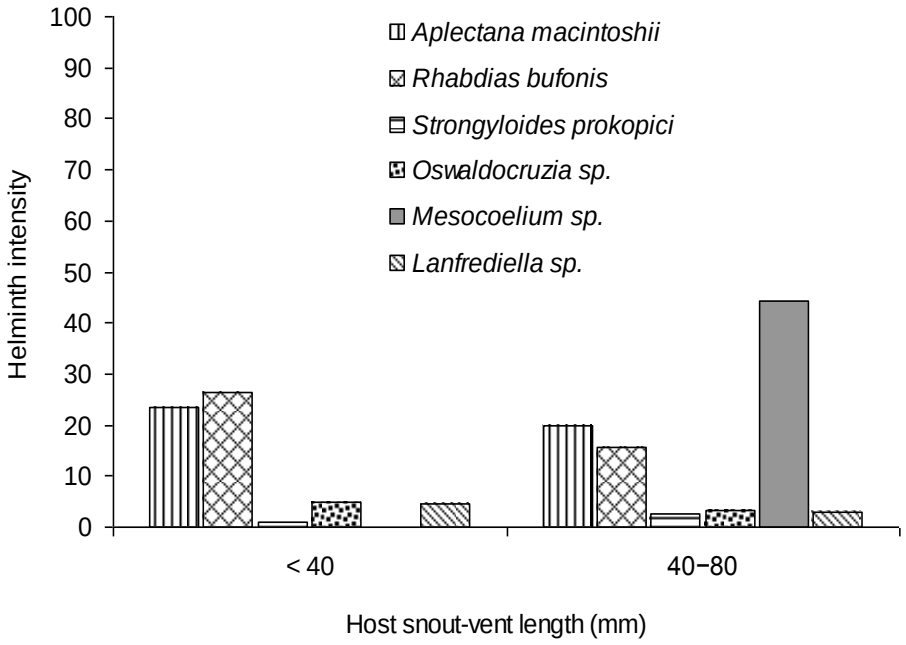

Figure 4: Intensity of helminth infections among *A. xeros* toads, *according to SVL, (< 40 mm: n = 15; 40-80 mm: n = 54).*