Gastrointestinal helminths of lizards (Reptilia: *Squamata*) from Egypt

Original Article

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ABSTRACT

Background: Despite the fact that Egypt contains a large number of lizard species, yet there is little knowledge recorded regarding the helminth fauna infecting this group of hosts. A parasitological study on gastrointestinal helminths of lizards was carried out during Summer (August) and Autumn (September and October), 2014 and 2015, respectively.

Objective: This study aims to update information on helminth parasites that infect lizards in Egypt.

Material and Methods: Seven species of lizards, *Pseudotrapelus sinaitus* Heyden, 1827; *Trapelus mutabilis* Merrem, 1820 (Agamidae); *Tarentola mauritanica mauritanica* Linnaeus, 1758 (Phyllodactylidae); *Tropiocolotes tripolitanus* Peters, 1880 (Gekkonidae); *Acanthodactylus boskianus* Daudin, 1802 (Lacertidae); *Chamaeleo africanus* Laurenti, 1768 (Chamaeleonidae); and *Varanus niloticus* Linnaeus, 1766 (Varanidae), were collected from several parts of Egypt. These hosts were dissected and examined for gastrointestinal helminth. The collected helminths were fixed in 70% ethanol, stained with carmine and examined by using an Olympus CX31 microscope and a Zeiss Stemi 2000-C microscope.

Results: The total prevalence of infection with helminths was 35.9%. The study recorded 16 species of helminths recognised as: six species of digenea [*Prosthodendrium obtusum* (Looss, 1896) Bhalerao, 1936, *Pleurogenoides tener* (Olsson, 1876) Travassos, 1921, *Anchitrema sanguineum* (Sonsino, 1894) Looss, 1899, *Cyclorchis varani* Price, 1936, *Mesostephanus milvi* Yamaguti, 1939 and *Haplorchis pumilio* (Looss, 1896) Looss, 1899], four species of cestodes [three adults, *Oochostrica mutabili* Morsy *et al.*, 2013, *Duthiersia fimbriata* Diesing, 1854 and one unidentified cestode and one tetrathyridia of *Mesocestoides* spp.] and six species of nematodes [*Parapharyngodon micipsae* Seurat, 1917, *Pharyngodon inermicauda* Baylis, 1923, *Abbreviata leptosome* Gervais, 1848, *Thubunaea pudica* Seurat, 1914, *Tanqua tiara* (von Linstow, 1879) Blanchard, 1904 and *Spinicauda sonsinoi* (Linstow, 1894) Travassos, 1920].

Conclusion: This study documents 10 new host and 10 new locality records in Egypt and distinguishes between studied material and reported descriptions. These findings add significantly to the present information on helminths infecting lizards in Egypt.

Keywords: Cestode, digenea, Egypt, helminths, lizards, nematode.

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INTRODUCTION

Egypt is in the north-eastern part of the continent of Africa with roughly one million square kilometres (1,019,600 km) of surface area. The country constitutes four major terrestrial physiographic regions (the Nile Valley, Western Desert, Eastern Desert, and Sinai) and two marine regions (Mediterranean and Red Sea)^[1,2], besides the River Nile. Lizards are some of the most successful wildlife hosts in Egypt^[2]. They have a large distribution range throughout different parts of Egypt. Only few studies on the helminth fauna of lizards have been previously reported^[3-9]. The aim of our survey is to identify and update the knowledge of the helminth fauna of lizards in Egypt.

MATERIAL AND METHODS

This parasitological study on gastrointestinal helminths of lizards was conducted during Summer

(August) and Autumn (September and October), 2014 and 2015, respectively. We collected seven species of lizards: Sinai agama, Pseudotrapelus sinaitus Heyden, 1827 from South Sinai (28°33'23"N, 33°58'28"E); Desert agama, Trapelus mutabilis Merrem, 1820 from El-Dabaa desert (30°1'36"N, 28°26'9"E) and Al Magarah, Al Hosnah, North Sinai (30°36'51"N, 33°43'18"E); Moorish gecko, Tarentola mauritanica mauritanica Linnaeus, 1758; Northern sand gecko, Tropiocolotes tripolitanus Peters, 1880 from 11th district near 6th October desert (29°53'18"N, 30°50′21″E); Bosk's fringe-fingered lizard or Bosc's fringe-toed lizard, Acanthodactylus boskianus Daudin, 1802 from Dahshour (29°47′44″N, 31°14′23″E); African chameleon, Chamaeleo africanus Laurenti, 1768 from Al Mansouria, Giza (30°7'49"N, 31°3'55"E); and Nile monitor, Varanus niloticus Linnaeus, 1766 from Lake Nasser, 120 km South of Aswan (23°25'25"N, 32°55'38"'E).

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PARASITOLOGISTS UNITED JOURNAL

Hosts were collected manually, euthanized and dissected. The body cavity of each host was examined and the oesophagus, stomach, small and large intestine of each lizard were examined separately for gastrointestinal helminths using a dissecting microscope (20X). For light microscopy, digenea and cestodes were flattened between two slides, fixed in 70% ethanol, stained with carmine, cleared in clove oil, and mounted in Canada balsam. Nematodes were fixed in 70% ethanol solution, cleared in lactophenol, and mounted in glycerol. Helminths were examined using an Olympus CX31 microscope, a Zeiss Stemi 2000-C microscope (4X, 10X, 40X, 100X) and identified according to Anderson *et al.*^[10], Bray *et al.*^[11], Gibson *et* al.^[12], and Khalil *et al.*^[13]. Taxonomy, identification and geographical distribution of the lizards were based on Baha El Din^[2] and Uetz *et al*.^[14].

Ethical Consideration: Approval to conduct this study was obtained from the Institutional Animal Care and Use Committee (ICUC), Faculty of Science, Tanta University.

RESULTS

A total of 167 lizards belonging to six families (Agamidae, Phyllodactylidae, Gekkonidae, Lacertidae, Chamaeleonidae and Varanidae) were dissected for detection of gastrointestinal helminths. The total prevalence of infection with helminths was 35.9%. Lizards were infected with sixteen species of helminths: six digenean species, four cestode species and six nematode species. Five lizard species were infected with two to seven helminth species. Data in table (1)

Helminths	Stage	Site of infection	Host	Prevalence (No. infected/ No. examined)	Intensity	Range
		Tr	ematode (Digenea)			
Pleurogenoides tener	Adult	SI	Chamaeleo africanus	14.29% (3/21)	66.33 ± 54.50	4-105
Anchitrema sanguineum	Adult	SI	Chamaeleo africanus	4.76% (1/21)	1 ± 0	1
Cyclorchis varani	Adult	GB, SI	Varanus niloticus†	31.25% (5/16)	4.40 ± 6.54	1-16
Prosthodendrium obtusum	Adult	SI	Chamaeleo africanus*	38.10% (8/21)	71.88 ± 78.00	3-210
Mesostephanus milvi	Adult	SI	Varanus niloticus*†	12.50% (2/16)	3.50 ± 3.53	1-6
Haplorchis pumilio	Adult	SI	Varanus niloticus*†	43.75% (7/16)	53.01 ± 17.06	1-134
			Cestode			
Oochoristica mutabili	Adult	SI	Pseudotrapelus sinaitus* Trapelus mutabilis† Tarentola m. mauritanica*†	30% (3/10) 27.78% (10/36) 9.38% (3/32)	9.00 ± 7.21 15.8 ± 16.06 1 ± 0	1-15 1-44 1
Duthiersia fimbriata	Adult	SI	Varanus niloticus	43.75% (7/16)	1.43 ± 1.13	1-4
Unidentified cestode species	Adult	SI	Varanus niloticus	12.50% (2/16)	5.5 ± 0.71	5-6
Tetrathyridium	Larva	SI	Pseudotrapelus sinaitus* Trapelus mutabilis*	10% (1/10) 5.56% (2/36)	49 26 ± 22.63	49 10-42
			Nematode			
Parapharyngodon micipsae	Adult	SI	Pseudotrapelus sinaitus*† Trapelus mutabilis*†	30% (3/10) 13.89% (5/36)	2 ± 1 8.2 ± 6.91	1-3 1-16
Pharyngodon inermicauda	Adult	SI	Tarentola m. mauritanica	34.38% (11/32)	5.27 ± 5.06	1-18
Abbreviata leptosoma	Adult	Stomach, SI	Pseudotrapelus sinaitus Trapelus mutabilis	30% (3/10) 5.56% (2/36)	4.33 ± 3.21 8.50 ± 7.78	2-8 3-14
Thubunaea pudica	Adult	Stomach, SI	Trapelus mutabilis	19.44% (7/36)	4.14 ± 5.79	1-17
Tanqua tiara	Adult	Embeded in stomach mucosa	Varanus niloticus*†	12.50% (2/16)	1.5 ± 0.71	1-2
Spinicauda sonsinoi	Adult	LI, rectum	Chamaeleo africanus	9.52% (2/21)	1 ± 0	1

Table 1: Prevalence, intensity (mean ± SD) and range of helminth species collected from lizards.

SI = small intestine; GB = gall bladder; LI = large intestine

* New host record.

† New locality record.

shows the infected host number for each parasite species, prevalence of infection, intensity and range as well as, 10 new host and 10 new locality records.

The Nile monitor, V. niloticus was infected with three digeneans: Cyclorchis varani Price, 1936 (Opisthorchiidae), Mesostephanus milvi Yamaguti, 1939 (Cyathocotylidae) and Haplorchis pumilio (Looss, 1896) Looss, 1899 (Heterophyidae); an adult cestode, Duthiersia fimbriata Diesing, 1854 (Diphyllobothriidae), a tetrathyridium metacestode (Mesocestoididae), an unidentified cestode species; and one nematode species, Tanqua tiara (von Linstow, 1879) Blanchard, 1904 (Gnathostomatidae). The Sinai agama, *P. singitus* was infected with an adult cestode, Oochoristica mutabili Morsy et al., 2013 (Linstowiidae), a tetrathyridium metacestode; and two nematode species: Parapharyngodon micipsae Seurat, 1917 (Pharyngodonidae) and Abbreviata leptosome Gervais, 1848 (Physalopteridae). The desert agama, T. mutabilis was infected with an adult cestode, *Oochoristica mutabili*, a tetrathyridium metacestode; and three nematode species: Parapharyngodon micipsae, Thubunaea pudica Seurat, 1914 (Physalopteridae) and Abbreviata leptosoma. The African chameleon, C. africanus was infected with three digeneans: Pleurogenoides tener (Olsson, 1876) Travassos, 1921 (Pleurogenidae), Anchitrema sanguineum (Sonsino, 1894) Looss, 1899 (Anchitrematidae) and Prosthodendrium obtusum (Looss, 1896) Bhalerao, 1936 (Lecithodendriidae); and one nematode species, Spinicauda sonsinoi (Linstow, 1894) Travassos, 1920 (Heterakidae). The Moorish gecko, T. m. mauritanica was infected with an adult cestode, Oochoristica mutabili; and one nematode species, *Pharvngodon inermicauda* Baylis, 1923 (Pharyngodonidae). The Northern sand gecko, Tropiocolotes tripolitanus Peters, 1880 and the Bosk's fringe-fingered lizard or Bosc's fringe-toed lizard, Acanthodactylus boskianus Daudin, 1802 were free from helminths.

The most frequently infected hosts were *P. sinaitus* and *V. niloticus* with 80% infection rate, followed by *T. m. mauritanica* (43.75%), *T. mutabilis* (38.88%) and *C. africanus* (38.09%). The prevalence of single infection with only one species of helminth was 20.96%, followed by double (8.98%), triple (2.40%), and quadruple (1.20%) infections.

DISCUSSION

Such variability in helminth community structure may result from variability in host diet, availability of intermediate host and host behavior which may have a direct effect on parasite transmission. In addition, the antagonistic response of intestinal infections of one group to others may affect the ability of some helminth to thrive even within their susceptible host. In our study, we found that *V. niloticus, T. mutabilis* and *C. africanus* are the most frequently infected hosts with multiple infections (digenea, cestodes and nematodes) at the same time. *Varanus niloticus* and *T. mutabilis* harbored six and four species of parasites respectively, the maximum number of species per host in each of them was four (in two hosts). *Pseudotrapelus sinaitus* harbored five species of parasites while the maximum number per host was three (in one host). *Chamaeleo africanus* harbored three species of parasites while the maximum number per host was three (in two hosts). *Tarentola mauritanica mauritanica* harbored two species, the maximum number per host was two (in three hosts).

The prevalence of single infection with only one species of helminth was highest than in mixed helminthic infections. This observation may reflect the antagonistic response of intestinal infections of one group to others and clarify the rarity of multiple infection. Saoud and Ramadan^[15] observed that infections with specific trematode genera was antagonistic to infection with other genera and that this antagonism appears to be dependent on the species of host involved.

Pleurogenoides tener is a common digenean of amphibians and some reptile species^[16]. In Egypt, only two species of *Pleurogenoides* (*P. tener* and *P. medians*) were reported^[6,16]. The present specimen was very similar to *P. tener* reported previously by Groschaft and Moravec^[6]. The present identification is based on the ratio between oral and ventral sucker, and the extent and shape of the excretory vesicle. In Egypt, few studies reported the presence^[6] and life cycle^[17] of *P. tener* in lizards.

Anchitrema sanguineum is a worldwide digenean reported from reptiles, bats and shrews as definite hosts and humans as accidental host^[11,18]. Only one specimen was found in our study. A full description was published by Saoud and Ramadan^[19] and El-Mahi^[20]. Chamaeleo chamaeleon Linnaeus, 1758^[6], Chamaeleo vulgaris (= C. chamaeleon) Khotenovsky, 1970 and Chamaeleo africanus Laurenti, 1768 (= C. basiliscus)^[21] were reported as final hosts in Egypt.

Cyclorchis varani is a digenean originally described from *V. niloticus* in Africa^[22]. The only species previously reported from Egypt was *C. fayoumensis* in the bile duct of the Egyptian Cobra, *Najahaje*^[23]. The collected specimens resembles description of *C. varani* by Price^[22]. The distinct constriction at the level of the ventral sucker, proximal part of uterus passing between ovary and testes or not, size of ventral sucker and testes arrangement are the significant characteristics to differentiate *C. varani* from *C. campula* and *C. amphileuous*^[22]. *Cyclorchis fayoumensis* is differentiated from the present specimen by the large size of the body, unequal intestinal caeca, deeply branched testes, dissimilarities in the position of the genital pore, the bifurcation of the main excretory duct, and the small sized egg^[23]. Lake Nasser, 120 km South of Aswan is a new locality record for *C. varani*.

Prosthodendrium obtusum is a digenean originally described from *Chamaeleo basiliscus* in Egypt^[6]. The genus *Prosthodendrium* includes five species that were reported from Egyptian bats^[24,25]. Reports on *Prosthodenduim* spp. from reptiles are few, only two were reported from Egypt^[6]. Aspinose body, size of ventral sucker being smaller than oral sucker, ovary formed of 7-9 lobes and as wide as prostate mass, two groups of vitelline glands anterior to testes and intestinal caeca are the diagnostic characters of *P. obtusum*. Human infection with *Prosthodendrium* spp. were recorded in Asia^[26,27].

Mesostephanus milvi is a digenean, first described from *Milvus migrans lineatus* in Japan^[28], while *M. milvi*, M. appendiculatus, M. fajardensis, M. dotternsi and M. *cubaensis* were recorded in Egypt^[29,30]. Our specimen differs from other species of the genus in having medium sized ventral sucker equal or sometimes smaller than the oral sucker; a muscular pharynx smaller than the oral sucker, short oesophagus; club-shaped cirrus pouch extending forward beyond the posterior testis to the right of it, distal part (post-prostatic) of the cirrus sac pocket about 1/5, 1/6 or 1/7 of the length of the cirrus; vitelllaria are intertesticular partially or fully overlapping gonads; vaginal sphincter is absent and uterus with 6–10 large eggs^[30-33]. This is the first report of *M. milvi* in *V. niloticus* in Egypt. To date, there are no records of *M. milvi* adult in monitors in the world.

Haplorchis pumilio is considered as a food-borne zoonotic digenean in many countries^[34,35] including Egypt^[36]. It completes its life cycle in fish of economic importance and in humans. The genus *Haplorchis* includes nine valid species; only three species, *H. pumilio, H. taichui,* and *H. yokogawai* were reported in Egypt^[37]. Species specific characteristics of *H. pumilio* are the structure and armature of the ventral sucker (crown of 32 to 40; I- or inverted capital V-shaped minute sclerites in *H. pumilio*, semi-lunar group of 12-16 long crescentic hollow spines in *H. taichui*, and 70-74 to numerous tiny spines in *H. yokogawai*)^[37,38]. Our study designates a new host and locality records.

Oochoristica mutabili is considered a heteroxenous cestode that requires a suitable intermediate host^[39]. The species of *Oochoristica* are cosmopolitan infecting more than 56 species of reptilian hosts, occasionally in mammals, and rare in amphibians^[40-43]. Genus *Oochoristica* includes about 88 species, only *O. mutabili*, *O. truncata*, and *O. fibrata* were previously recorded in Egypt^[9,41,44]. Our specimen is characterized by a scolex with circular suckers (105-150 *u*), distinct neck, 20-47 testes arranged in one cluster, and bilobed ovary. These morphological characteristics and measurements

are similar to those reported by Morsy *et al.*^[44] in their description. *Pseudotrapelus sinaitus* and *T. m. mauritanica* are new host records for *O. mutabili*, and Al Magarah in North Sinai, El-Dabaa desert, and 6^{th} October dessert near Giza are new geographical localities.

Tetrathyridium is the larval stage (metacestode) of the genus Mesocestoides. Species of Mesocestoides are unique among Cyclophyllidea in requiring three hosts in the life cycle^[12,45], the possibility of asexual reproduction of the tetrathyridial metacestodes by longitudinal fission^[46], and certain anatomical features that include the median ventral position of the genital atrium and the bipartite vitelline glands. Specific identification was not possible because of the absence of mature and gravid segments for this larval stage. Adult of *Mesocestoides* tapeworms have been reported previously in humans in Korea^[47], Japan^[48], China^[49], USA^[50] and Paraguay^[51]. *Cercopithecus albigularis* Sykes, 1831^[52], Acomys dimidiatus Cretzschmar, 1826^[53], Acomys cahirinus É. Geoffrey, 1803, Acomys russatus Wagner, 1840, Dipodillus dasvurus Wagner, 1842, D. amoenus amoenus de Winton, 1902, Meriones crassus Sundevall, 1842, M. tristrami Thomas, 1892^[54], M. Shawi isis Thomas, 1919, Pachyuromys duprasi natronensis de Winton, 1903, Gerbillus gerbillus gerbillus Olivier, 1801 and *G. pyramidium pyramidium* I. Geoffroy, 1825^[20,55] were reported as hosts of tetrathyridium in Egypt. Our study is the first host record for tetrathyridium in P. sinaitus and T. mutabilis.

Duthiersia fimbriata cestode was described by Diesing from *V. niloticus* in Africa^[56,57]. *Duthiersia* was reported in two forms: an Asiatic and an African form. The two forms can be morphologically distinguished on the basis of presence or absence of posterior pores in the scolex^[57]. Few reports were recorded on African *D. fimbriata*^[58,59]. Woodland^[56,59] and Elmahy and Harras^[60] gave full descriptions for *D. fimbriata*.

Parapharyngodon micipsae is an oxyurid nematode characterized by four pairs of caudal papillae, presence of narrow weakly developed lateral alae, spicule of male with pointed or sharp tip, echinate cloacal lip present and the ovaries reaching anteriorly to the oesophagus base forming prominent coils around it^[8,61]. This parasite is widely distributed^[62]. *Parapharyngodon* includes more than 59 species^[63,64]. *Parapharyngodon micipsae, P. bulbosus* Linstow, 1899 and *P. sceleratus* Travassos, 1923 are the only species known from Egypt^[8,65]. The recording of *Parapharyngodon micipsae* in our study represents new host and locality documentation.

Pharyngodon inermicauda is an oxyurid nematode originally described from *Tarentola annularis* Geoffroy Saint-Hilaire, 1827 in Cairo, Egypt^[3]. *Pharyngodon inermicauda* and *P. mamillatus* Linstow, 1897 are the only reported species in Egypt^[8]. The identification is based on the absence of male spicule, no inflation at

the junction between lateral and caudal alae in male *P. inermicauda*, the anterior pair of postanal papillae not forked, lack of spines in the female caudal filament, and the pointed egg^[3,66]. Members of Oxyurida are monoxenous^[67].

Abbreviata leptosoma is a spirurid nematode, first described by Chabaud^[68] in the gut of *Uromastiz acanthinurus* in Algeria. *Abbreviata* spp. are cosmopolitan parasites with poorly recognized life cycle; final hosts comprise most vertebrates including amphibians, reptiles, birds and mammals, and intermediate hosts are invertebrates^[68,69]. Characteristics of both male and female *A. leptosoma*^[68] include the cephalic denticulation with 5-8 elements between submedian teeth and lateral tooth, absence of denticulate crest at the dorsal and ventral angles of the mouth, presence of two branched uteri, and unequal dissimilar spicules.

Thubunaea pudica spirurid nematode has been previously reported in Egypt from different reptilian hosts; *Scincus scincus*^[7], *Psammophis schokari*^[70], *Agama bibroni, Lacerta lepida, Psammodromus algirus,* and *Eumeces algeriensis*^[71]. Genus *Thubunaea* includes more than 23 species, two of them (*T. pudica* and *T. dactyluris*) were previously reported in Egypt^[70,72,73]. *Thubunaea pudica* has simple equal lips, three teeth on each lip without ring of papillae or cuticular groove subtending the lips, short laterally compressed vestibule, male caudal papillae (about 32 papillae), and two subequal stout spicules^[70,74]. Cockroaches and crickets are reservoir hosts of larval stage of *Thubunaea* spp.^[75,76], while reptiles were reported as final hosts^[5].

Tanqua tiara belongs to genus *Tanqua* R. Blanchard, 1904 of the family Gnathostomatidae. Reptiles (mainly Monitor lizards, Varanidae) and amphibians were reported as definite hosts for this genus^[77]. There are ten described species of *Tanqua*, only one species (*T. tiara*) was reported in Africa. It is differentiated from other species on the basis of the presence of head bulb divided into four swellings each with a ballonet, non-retractile head, uterus with 4 branches (3 extend anterior and one posterior), and vulva in the last quarter of the body^[77,78]. Only three females were collected. Our study represents the first record of *T. tiara* in Egypt.

Spinicauda sonsinoi is an ascarid nematode belonging to family Heterakidae. The species was originally described from *Laudakia stellio* in Egypt^[79]. Genus *Spinicauda* includes 17 species, of which only *S. sonsinoi* and *S. grimmae* were previously reported in Egypt^[4,8,9,70]. The length of male spicule, the number of caudal papillae, presence or absence of female caudal papillae, and presence or absence of a gubernaculum are the main characteristics to differentiate between species^[79,80]. The development of *Spinicauda* is monoxenous^[67]. *Chamaeleo africanus* is a new host record.

Goldberg and Bursey^[81] reported that the difference in prevalence and intensity of helminth infection in reptiles is related to the attribution of dietary variation of host species. This is well illustrated in table (1), since infection of helminths requires various intermediate hosts which varies with the feeding habits and environment of the final hosts.

Chai^[27] marked 70 species of digenetic trematodes that infect humans. He classified them as blood flukes, liver flukes, lung flukes, and intestinal flukes based on their habitat in the definite host. *Haplorchis, Prosthodendrium* and *Mesocestoides* tetrathyridia reported in the present study are considered human infecting intestinal flukes^[26,27].

In conclusion, our study documented ten new host and ten new geographical locality- records in Egypt. These findings add significantly to the present information on helminths infecting lizards and their potential contribution to public health.

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Author contribution: RA Elmahy initiated the research idea, designed the study, identified the helminths, collected the references and wrote the manuscript. SF Harras contributed in practical work in Central laboratory in Zoology Department, and shared RA Elmahy in designing the study and identification of helminths.

Conflict of interest: Authors confirm that there are no known conflicts of interest associated with this study.

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