

**New host and locality record of *Parapharyngodon japonicus* (Nematoda: Oxyuroidea) from the Egyptian changeable lizard *Agama mutabilis* (Agamidae): A light and scanning electron microscopy**

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**Summary**

*Parapharyngodon* (Oxyurida) is a lizard gastrointestinal nematode parasite with a life cycle including lizards as main hosts. However, some species are known to parasitize anurans. In the present study, *P. japonicus* isolated from the large intestine of the Egyptian changeable lizard, *Agama mutabilis* was described and illustrated. Forty five specimens of these animals were collected from south Sinai desert, Egypt during the period from May to September 2017. After necropsy, the body was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was removed. The esophagus, stomach, small and large intestines were examined separately for helminthes. The recovered nematodes were examined by light and scanning electron microscopy. Thirty six specimens (80.0 %) were found to be naturally infected. The parasite was robust with prominent cuticular transverse annulations. Mouth surrounded by three bilobed lips, each with tiny labial papillae. Three pairs of caudal papillae were observed in male worms; 1 pair precloacal, 1 pair sublateral in cloacal opening line, 1 pair in proximal region of caudal appendage on its narrowed point. The posterior extremity beard dorsally directed caudal appendages. Females were with a conical posterior end terminated at a terminal spike. Ovaries reached esophageal isthmus but not wrapped around corpus. The parasite recorded was compared morphologically and morphometrically with the most similar species, it was found that it was most similar to *P. japonicus* with new host and locality records.

**Keywords:** *Parapharyngodon japonicus*; Nematoda; *Agama mutabilis*; Agamidae; light and scanning electron microscopic study

**Introduction**

The taxonomic status and validity of the genus *Parapharyngodon* have been questioned almost since its proposal by Chatterji (1933). Baylis (1936) considered it to be a synonym of the genus *Thelandros* Wedl (1861) (see Adamson, 1981; Adamson & Nasher, 1984; Bursey & Goldberg 1999, 2005). Later on, Freitas (1957) reinstated the genus *Parapharyngodon* which was accepted by several authors (Skrjabin *et al.*, 1960; Baru & Coy-Otero, 1969; Baru, 1973; Sharpilo, 1976; Baker, 1987; Castazo-Fernan-

dez *et al.*, 1987; Ashour *et al.*, 1994; Bursey & Goldberg, 2007a). Adamson (1981) re-established *Parapharyngodon* based on dietary habits of the host, morphology of the male genital cone and female tail and eggs. Males of *Parapharyngodon* spp. lack conical-shaped genital area and accessory piece. They have mammilliform papillae surrounding the more-or-less terminal anus and sub-terminal dorsally directed tail. Whereas males of *Thelandros* have a genital cone with pendulant papillae outside this cone, an accessory piece as well as terminal posteriorly directed tail is present in some species (Bursey *et al.*, 2013; Pereira *et al.*,

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2017). Females of *Parapharyngodon* spp. possess a conical tail terminated in a short stout spike, eggs with sub-terminal operculum in the early stages of cleavage when released (Burseley *et al.*, 2004; Anjum *et al.*, 2013). Females of *Thelandros* have various tail morphologies, eggs with a terminal operculum larvated when released (Burseley *et al.*, 2013; Pereira *et al.*, 2017). More than 40 well described species are assigned to the genus *Parapharyngodon* according to Burseley and Goldberg (2007a,b) and Gupta *et al.* (2009). The Egyptian changeable lizard, *Agama mutabilis* Merrem (1820) is widespread across northern Africa, occurring

from Western Sahara, Mauritania and Morocco east to Egypt and Sudan. This is an active predator exhibits a diurnal behavior and insectivorous including beetles, caterpillars and ants in diets and in some instances it has been known to eat large migratory locusts as they pass through its habitat. The present study reports the finding of *P. japonicus* in a new host, *Agama mutabilis* and locality, Egypt, including detailed morphological and morphometric characterization of this species using light and scanning electron microscopy (SEM).

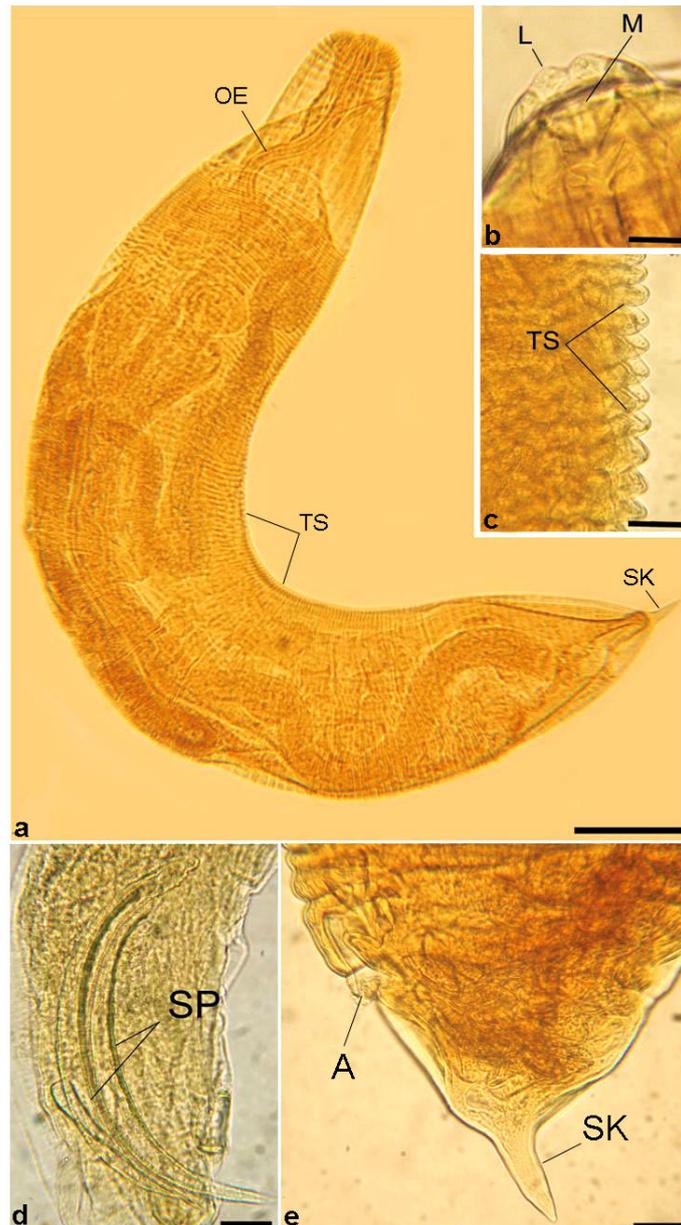


Fig. 1. (A – E): Photomicrographs of *P. japonicus* showing: A – Females, lateral view with terminal spike (SK), TS transverse cuticle striations. B – Details of the cephalic end, mouth opening (M) surrounded by three bi-lobed lips. C – Transverse cuticle striations (TS). D – Posterior end of male with two spicules (SP). E – Posterior end of female terminated at a caudal spike (SK). Scale bars: A = 300  $\mu$ m; B, C = 10  $\mu$ m; D = 100  $\mu$ m; E = 50  $\mu$ m.

## Materials and Methods

Forty-five specimens of the mean snout-vents *Agama mutabilis* (length up to 9.4 cm) were collected by hand or noose from South Sinai desert, Egypt during the period from May to September 2017. Animals were subjected to euthanasia using 20 % benzocaine gel (Anbesol, Pfizer, Inc., New York). Each specimen was subsequently necropsied and all organs were examined for helminthes using a ZEISS Compact Greenough stereomicroscope (Model Stemi 305). All animal procedures were carried out according to the regulatory laws regarding experimental Animal Ethics Committee. Nematode worms were isolated from host intestines, heat fixed in 10 % neutral buffered formalin for 15 min and then preserved in 70 % ethanol in 5 % glycerol solution to avoid sudden drying. Finally, samples were transferred to lactophenol for clearance. The prepared samples were examined using differential interference contrast (DIC) light microscopy with digital image analysis system (analysis auto 5.0). Drawings were made with the aid of a camera lucida. Measurements were in micrometer unless otherwise stated. For SEM, samples were fixed in 4 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), washed in the same buffer, and dehydrated in a graded alcohol series. Samples were then processed in a critical point drier "Bomer-900" with freon 13, sputter-coated with gold-palladium in a Technics Hummer V, and finally examined with a Jeol scanning electron microscope (Model JSM7610F).

## Ethical Approval and/or Informed Consent

All animal procedures were carried out according to the regulatory laws regarding experimental Animal Ethics and Use Committee.

## Results

Thirty six out of 45 (80.0 %) specimens of the Egyptian changeable lizard, *A. mutabilis* were infected with nematode parasites isolated from their intestines. Worms were examined morphologically by light and SEM.

### *Parapharyngodon japonicus* Houttuyn (1782)

Description based on 13 specimens (Figs. 1 – 3): Robust cylindrical nematodes with prominent cuticle annulations from beginning of the esophagus to the anal opening. Oral opening was triangular, surrounded by three bilobed lips. Each lobe beard tiny labial papillae. Buccal capsule absent. Sexual dimorphism evident, females larger and more robust than males. Lateral alae present in males, but absent in females. Males without caudal alae, caudal filament subterminal and directed dorsally. Females with conical tail terminated in a short stout spike.

Male: Small fusiform nematodes measured 1735 – 2986 ( $2280 \pm 10$ )  $\mu\text{m}$  long, 385 – 490 ( $438 \pm 11$ )  $\mu\text{m}$  wide at the level of the excretory pore. Lateral alae began at the level of esophageal isthmus. Total esophagus length 290 – 460  $\mu\text{m}$  ( $388 \pm 7$ ). Bulb was

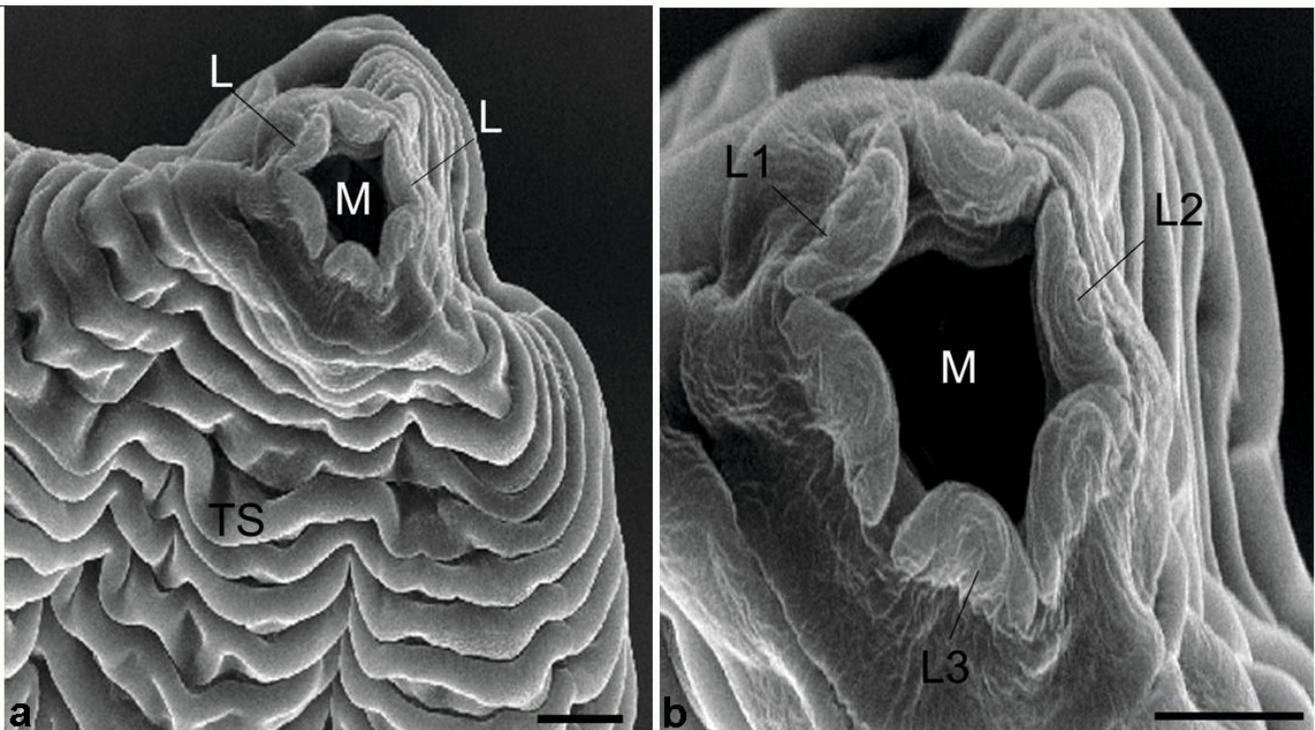


Fig. 2. (A, B): Scanning electron micrographs showing apical views of the cephalic end, L symbolized for the three bilobed lips (L1, L2, L3) surrounding mouth (M); TS for transverse cuticle striations. Scale bar = 10  $\mu\text{m}$ .

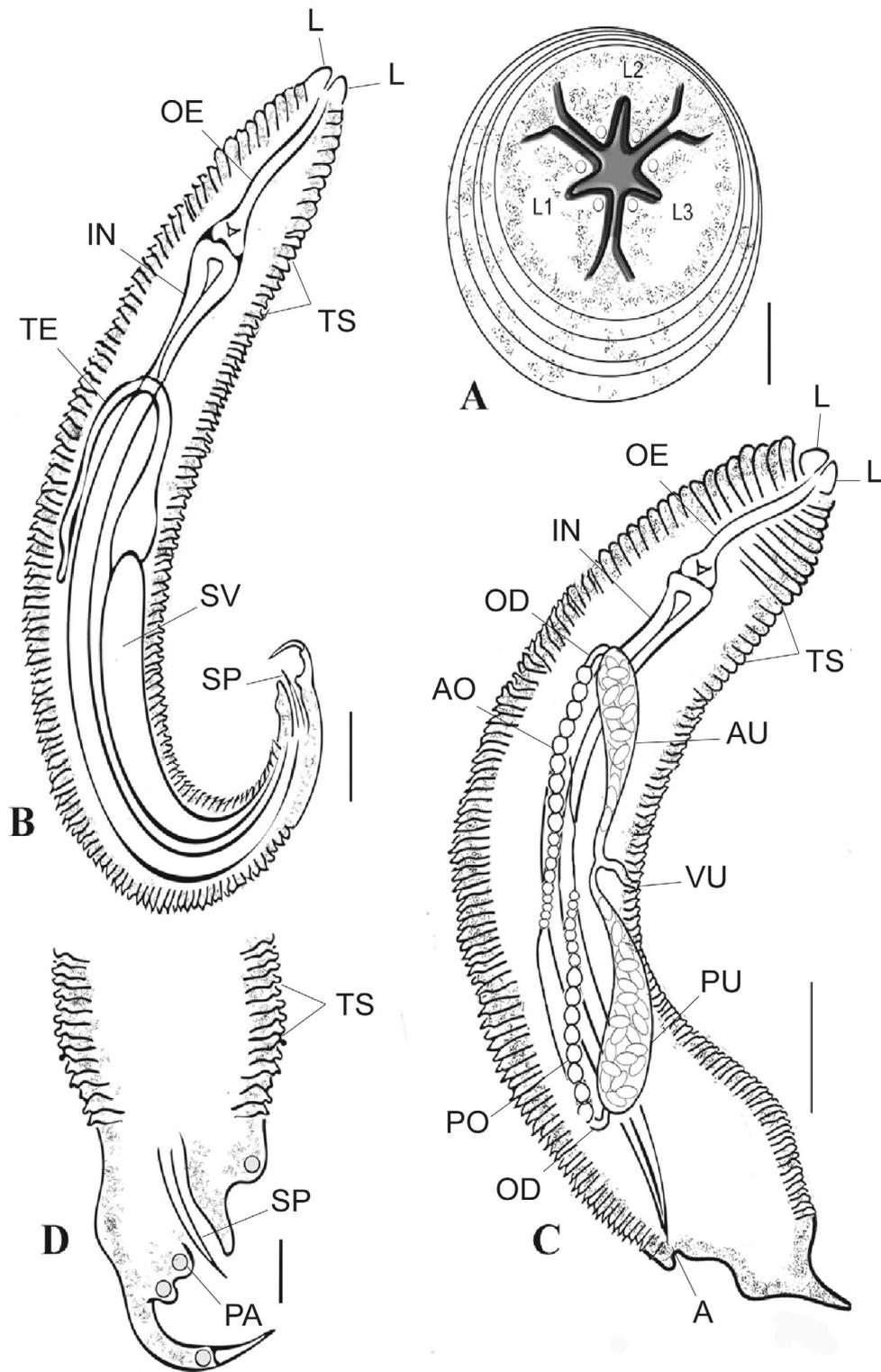


Fig. 3. Line drawings of *P. japonicus*. A, cephalic end of paratype female, apical view. B, Holotype male, lateral view. C, paratype female, lateral view. D, Posterior end of male, lateral view. L lips; OE oesophagus; IN intestine; TS transverse striations; TE testis; SV seminal vesicle; SP spicule; AO anterior ovary; PO posterior ovary; OD oviduct; AU anterior uterus; PU posterior uterus; A anus; PA caudal papillae. Scale bars: A = 10  $\mu$ m; B = 200  $\mu$ m; C = 300  $\mu$ m; D = 100  $\mu$ m.

Table 1. Comparative measurements between the present species and members of family Pharyngodonidae previously reported from lizards in Egypt, measurements in mm, otherwise stated.

Species	Host	locality	length	width	Spicule (mm)	Cloacal lip	Ovary	Egg size	Reference
<i>T. alatus</i> (Wedle, 1862)	<i>Agama stellio</i>	El Quseima, North Sinai	5.94	0.44	-	-	-	14X(72 – 84) $\mu$ m	Edward A. Belle, 1954
<i>T. kasauli</i> (Chatterji, 1936)	<i>Agama stellio</i>	El Quseima, North Sinai	Female: 5.23 Male: 3	Female: 0.35 Male: 0.31	100 $\mu$ m	-	-	128X68 $\mu$ m	Edward A. Belle, 1954
<i>T. bubosus</i> (Linstow, 1899)	<i>Agama – Scincus</i>	Berg el Arab (Agama), Wadi Faran, S.Sinai (Scincus)	Female 3.8 – 4.61 Male: 2.6	-	74 – 79 $\mu$ m	-	Prebulbar	97X57 $\mu$ m	Edward A. Belle, 1954
<i>T. micipsae</i> (Seurat, 1917)	<i>Chalcides sepioides</i>	Zawiet, Abu Musa 1lam, Giza	Female : 6.90 – 7.0 Male: 2.30	Female: 8.0 Male: 0.17	74 $\mu$ m	-	Postbulbar	50X90 mm	Edward A. Belle, 1954
<i>T. cameroni</i> Edward A. Belle 1954	<i>Chalcides sepioides – Scincus</i>	Berg el Arab, W. Desert, Korn. Aushdim, Faiyam Province	Female:3.28 Male: 2.34	Female: 0.31 Male:0.20	72 $\mu$ m	-	Postbulbar	79 – 95 X 52 – 54 $\mu$ m	Edward A. Belle 1954
<i>T. kuntz</i> (Edward A. Belle 1954)	<i>Agama</i>	Wadi Faran, S. Sinai	Female:3.20 – 4.20 Male:2.23 – 2.65	Female:0.39 Male:0.20	50 $\mu$ m	-	-	100X58 $\mu$ m	Edward A. Belle, 1954
<i>Thelandros</i> sp.	<i>Chalcides ocellatus</i> <i>Chalcides sepioides</i>	S.Sinai	Female: 2.65 – 3.85 Male:1.85 – 3.02	Female: 0.36 – 0.46 Male:0.17 – 0.25	-	-	Prebulbar	78 – 84 x 51 – 68 $\mu$ m	Rabie et al, 2012
<i>Pharyngodon hindlei</i> (Thapar, 1925)	<i>Eumeces schneiderii</i>	Berg El Arab, W. Desert.	Female:4 – 5 Male:2.5 – 0.3	Female:0.4 – 0.47 Male:0.16	0.045 – 0.054	-	-	140X42 – 51	Edward A. Belle, 1954
<i>Pharyngodon extenuates</i> (Rudolphi, 1819)	<i>Acanthodactylus</i>	Baltim, Fouadiya Province,	Female:5 – 6.7	Female:0.36	-	-	-	144X33 – 36	Edward A. Belle, 1954
<i>Pharyngodon inermicauda</i> (Baylis, 1923)	<i>Tarentola mauritanica</i>	Abu Rawash	Femal:3.60 – 3.81 Male:1.46 – 1.84	Female:0.326 – 0.340 Male:0.095 – 0.163	absent	-	In the middle third	0.150 – 0.165 X 0.042 – 0.51	Moravec et al. , 1987
<i>Pharyngodon mamillatus</i> (Linstow, 1897)	<i>Chalcides ocellatus</i>	Abu Rawash	Female:3.26 – 3.63 Male:1.07 – 2.23	Female: 0.340 – 0.394 Male:0.109 – 0.204	0.033 – 0.045	-	In the middle third	0.135 – 0.144 X 0.036 – 0.042	Moravec et al. , 1987
<i>P. bubosus</i> (Linstow, 1899)	<i>Chalcides ocellatus</i>	Abu Rawash	Female:2.86 – 4.28 Male:2.14 – 2.46	Female:0.340 – 0.449 Male:0.231 – 0.236	0.051 – 0.063	smooth	Postbulbar	90 – 99X54 – 57	Moravec et al. , 1987
<i>P. micipsae</i> (Seurat, 1917)	<i>Scincus scincus</i>	Abu Rawash	Female:4.46 – 0.6.77 Male:1.71	Female:0.503 – 0.830 Male:0.095mm	88 $\mu$ m	echinate	prebulbar	91 X 50	Moravec et al. , 1987
<i>P. japonicus</i> (Present study)	<i>Agama mutabilis</i>	S. sinai	Female: 2150 – 3690 $\mu$ m Male:1735 – 2986 $\mu$ m	Female: 386 – 630 $\mu$ m Male:385 – 490 $\mu$ m	381 – 590 $\mu$ m	smooth	Postbulbar	76 – 120 $\mu$ m	

96 – 161 (140 ± 6) µm long and 65 – 105 (86 ± 5) µm wide. Nerve ring and excretory pore were 60 – 118 (70 ± 9) µm and 40 – 76 (46 ± 6) µm from the anterior end respectively. Testis reflexed posteriorly behind esophagus and the vas deferens separated from testis by a narrow tube. Three pairs of caudal papillae; 1 pair pre-cloacal, 1 sublateral pair in cloacal opening line, 1 pair in proximal region of caudal appendage on its narrowed point. Spicules were 381 – 590 (550 ± 11) µm long. Posterior extremity of the body beard dorsally directed caudal appendages, terminated at a thin tip, 18 – 23 µm (20 ± 5) long.

**Female:** Body length 2150 – 3690 (2450 ± 17) µm long x 386 – 630 µm (510 ± 12) wide at the level of vulva. Esophagus length was 282 – 460 (375 ± 8) µm long; Bulb length 89 – 170 (153 ± 7) µm; bulb width 85 – 120 (96 ± 10) µm. Nerve ring and the excretory pore were at 64 – 110 (81 ± 10) µm and 42 – 86 (56 ± 7) µm from the anterior end respectively. Uteri were Amphidelphic and divergent; the anterior uterus directed anteriorly while the posterior uterus was posteriorly directed and joined at the mid body. Ovaries reached esophageal isthmus but not wrapped around corpus. Eggs were oval, slightly flattened, thin-shelled and with subterminal operculum. Asymmetrical eggs extracted from ovjector in the early stages of cleavage. The posterior end of female was conical with terminal stout spike 76 – 120 (90 ± 13) µm long.

#### Taxonomic Summary

Species: *Parapharyngodon japonicus* Houuttuyn (1782)

Host: *Agama mutabilis* (Family: Agamidae)

Infection Site: Small intestine.

Locality: South Sinai, Egypt.

Prevalence: 36 out of 45 (80.0 %) specimens were infected

Deposition: Permanent slides of paratype female and holotype male were deposited at the Parasitology Division, Zoology Department Museum, Faculty of Science, Cairo University, Egypt.

## Discussion

The nematode fauna of Egyptian reptiles has received little attention in recent years. The only studies include those of Seurat (1917), Baylis (1923) and Moravec *et al.* (1987); they described several species of nematodes from African reptiles, mostly of the genera *Agama* and *Scincus* (Table 1). The recorded species in the present study was assigned to the genus *Parapharyngodon* according to the key published by Bursey and Goldberg (1999) where species of *Parapharyngodon* are distinguished on the basis of the pattern of caudal papillae, morphology of the anterior cloacal lip, the location of the ovary, and geographical distribution. Worldwide, there are currently 83 nominal species have been assigned to *Parapharyngodon*, with sufficient morphological features for both male and female nematodes (Bursey & Goldberg, 2015; Ramallo *et al.*, 2016). The parasite recorded herein can be differentiated from *Thelandros* sp. based on egg development during posture and the posterior end morphology in both sexes (Bursey & Goldberg, 2005). *Parapharyngodon* sp. males do not have a conical-shaped

genital area, or an accessory piece. They have mammilliform papillae and a dorsal subterminal tail. Males of *Thelandros* sp. have a conical-shaped genital area with papillae disposed outside this cone (Bursey & Goldberg, 1999; Bursey *et al.*, 2013). Females of *Parapharyngodon* sp. generally have a cone shaped tail with a thick pointed end, like a spike, eggs with a subterminal operculum that are un-cleaved, or in early stages of cleavage when released. In contrast, *Thelandros* sp. females have diverse tail morphology, eggs with terminal operculum, larvae are fully developed when they are released (Ramallo *et al.*, 2016; Bursey *et al.*, 2013; Velarde-Aguilar *et al.*, 2015). *Parapharyngodon japonicus* recovered from *Onychodactylus japonicus* by Bursey and Goldberg (1999) in Japan is most similar to the present nematode isolated. Where both share the presence of postbulbar ovarian coils, the tail of female terminated at a small spike, eggs are thin-walled and the anterior cloacal lip is smooth. They resemble *P. tyche* in the presence of smooth anterior cloacal lip, ovary is postbulbar, and the eggs are thin-walled and oval in outline, the female spike is small and uterus is thick-walled. They differ from each other in that the spicules in *P. japonicus* male is half the length of those in *P. tyche*, and the lateral alae of *P. japonicus* end abruptly about 80 µm anterior to the cloaca, whereas in *P. tyche*, the lateral alae continue to the end of the body. All of the nematode species of family Pharyngodonidae reported from lizards in Egypt were compared (Table 1); two of them are from the same genus, *P. bulbosus* (Linstow, 1899) by Moravec *et al.* (1987) from *Chalcides ocellatus* in Egypt and *P. micipsae*. *P. bulbosus* differ from the present species in that tails of females which is conical without distinct caudal spike and ovarian coils not reaching anterior level of esophagus; while *P. micipsae* differ by the presence of postbulbar ovaries in females and their anterior ends forming prominent coils around the base of esophagus while their males have 4 pairs of caudal papillae and echinate anterior cloacal lip. Moravec *et al.* (1987) isolated a nematode *P. aegyptiacus* which further has since been transferred to *Skrjabinodon inglis* by Moravec and Barus (1990).

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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