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Supplementary studies of *Pleurogenoides medians* (Digenea: Lecithodendriidae) infecting the Marsh frog *Rana Ridibunda* (Amphibia: Ranidae) in Egypt

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ABSTRACT

Pleurogenoides medians, a digenean lecithodendriid trematode, parasitize numerous aquatic vertebrate species including frogs, freshwater fish, urodeles and anurans. In the present study, a total of 190 out of 300 (63.33%) marsh frogs *Rana Ridibunda* were found to be infected with this digenean parasite. The highest percentage of infection was recorded in winter reaching 93.33%, and the lowest value was recorded to be 6.66% during summer. Prevalence and intensity of infection were positively correlated with the host size. Host sex showed no effect in this respect. Morphological studies based on light and scanning electron microscopy revealed that the adult worms characterized by small body size measured 1.980–2.430 (2.205 ± 0.1) mm long and 0.870–1.20 (9.87 ± 0.01) mm wide with spines of similar size distributed all over the body surface; oral sucker is sub-terminal and measured 0.180–0.230 (0.203 ± 0.01) mm long and 0.120–0.180 (0.150 ± 0.01) mm wide; ventral sucker is smaller than the oral sucker, post-ovarian located at 1/3 level from the anterior end and measured 0.080–0.102 (0.090 ± 0.001) mm long and 0.100–0140 (0.120 ± 0.001) mm wide; two symmetrical testes were located near the cecal termination on both sides of the ventral sucker, measured 0.153–0.193 (0.176 ± 0.01) mm long and 0.160–0.192 (0.175 ± 0.01) mm wide; pre-acetabular ovary was present and measured 0.130–0.150 (0.140 ± 0.01) mm long and 0.100–0.130 (0.120 ± 0.01) mm wide; vitellaria are extra-caecal and extended from the level of the pharynx to a level slightly beyond the ovary. By comparing the recovered parasite with different species of the same genus from different hosts having different localities, it was found that the present species morphometrically more or less different from the comparable species and the only similar species was *P. medians* described previously from the common toad *Bufo bufo* by having all similar characteristic features. In addition, the present study was considered as the first report for the occurrence of this lecithodendriid species infecting the marsh frog in Egypt.

1. Introduction

Frogs are widespread and likely to numerous aquatic vertebrates representing a very important link in food chains and, generally, they occupy an important position in many biocoenoses (Abu Ahmed and Begum, 2006). Like other vertebrates, frogs serve as the most preferred vertebrate hosts for a considerable range of metazoan parasites (Darrel, 2013), and their normal growth was inhibited if they had been heavily infected with *endo*-parasites (Mohammad et al., 2010). Little is known about the amphibian parasites in the Middle East. Madi (1976) isolated four trematodes *Gorgoderina vitelliloba*, *Prosotocus confusus*, *Pleurogenoides tacapensis* and *Haematolechus variegatus* from *Rana bedriagae* and *Bufo viridis* in Azraq Oasis, Jordan. Hassan (1988) recovered two trematodes from *Bufo regularis* in Egypt. Fernando (1989) isolated two adult digenetic trematodes *Pleurogenoides stromi*, *Pleurogenoides compactus* from *Rana ridibunda* in Saudi Arabia. Vojtkova and Roca (1995) described *Polystoma macrocnemis* from the long legged wood frog *Rana macrocnemis* in Turkey. Yildirimhan et al. (1997) isolated two species of trematodes *Pleurogenes caliveger* and *Gorgoderina vitelliloba*, and one monogenean species *Polystoma* sp. from *Rana macrocnemis* in Turkey. Furthermore, Yildirimhan (1999) reported *Polystoma viridis*, *Proteocephalus* sp., *Nematotaenia dispar* as well as other nematodes

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and one acanthocephalan from *Bufo viridis* in Turkey. In addition, seasonal studies of the helminth communities of amphibians are few; however, Bolek and Coggins (2000, 2001) studied the seasonal occurrence and community structure of helminth parasites of the eastern American toad *Bufo americanus americanus* and green frog *Rana clamitans melanota* in the USA.

Therefore, the present study aimed to record and described digenean parasite *Pleurogenoides medians* for the first time from the Marsh frog *Rana Ridibunda* in Egypt using morphological and morphometric analysis by means of light and scanning electron microscopy to open the way for more detailed investigations of trematodes of frogs from Egypt, in order to help in the elucidation of the morphology of adult worms and to establish the special adaptations of individual species within their host. Additionally, study the possible effects of intrinsic factors (host sex, weight and age) and an extrinsic factor (season) on the prevalence, mean abundance and intensity of this digenean parasite.

2. Materials and methods

2.1. Samples collection and parasitological examination

A total of 300 live adult specimens of the Marsh frog *Rana Ridibunda* (Family: Ranidae) were randomly collected during the period of February 2015–January 2016 from different localities at El-Giza province, Egypt. All collected samples were transported to the Laboratory of Parasitology Research at Zoology Department, Faculty of Science, Cairo University, Egypt; to be placed for 12 h at 23 °C in a plastic food container containing 1.3% v/v of Simplified Amphibian Ringer (SAR) to distilled water. Identification of frog specimens was carried out according to Frost (2006). Total length (mm), wet weight (g) and sex of each frog was determined. Within 48 h, frogs were overdosed in ether-filled glass containers. The body cavity was opened by a longitudinal ventral incision. The alimentary canal was excised and separated into stomach, small intestine, large intestine and rectum. The contents of each part and other organs (lungs, liver, gall bladder, kidneys, and urinary bladder) were each mixed with 0.5% saline solution and poured into petri-dishes for examination under a stereomicroscope for helminth parasites. In addition, muscles, plus portions of peritoneum and spinal cord, were teased out with needles and examined under a stereomicroscope. The recovered trematode parasites were removed, counted, fixed in Davidson's AFA (acetic acid, formalin, alcohol) and preserved in 70% ethanol, stained in semichon's aceto–carmine, dehydrated, cleared in xylol, and mounted on slides in Canada balsam. Slides were then incubated at 60°C for 24 h to driving the air bubbles according to Schmidt (1992). Taxonomic identifications of the recovered digenean worms were based on Yamagust (1971). Photomicrographs were produced using a Zeiss Axiovert 135 light microscope equipped with a Canon digital camera. Parasite prevalence, abundance and mean intensity (±SE) were calculated according to Bush et al. (1997). For scanning electron microscopy, recovered parasites were fixed at 3% glutaraldehyde, washed in sodium cacodylate buffer, dehydrated in a graded series of ethanol, infiltrated with amyl acetate. After passing through an ascending series of Genesolv D, they were processed in a critical point dryer "Bomer-900" with Freon 13, sputter-coated with gold–palladium in a Technics Hummer, examined and photographed under an Etec Autoscan at 20-kV Jeol scanning electron microscope in Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt. Measurements were taken with an Olympus ocular micrometer and expressed in millimeter as a range followed by mean ± SD in parentheses; otherwise were stated. Drawings were made with the aid of Camera Lucida.

2.2. Ethical considerations

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by Institutional Animal Care and Use Committee (IACUC) at Zoology Department in Faculty of Science, Cairo University, Egypt.

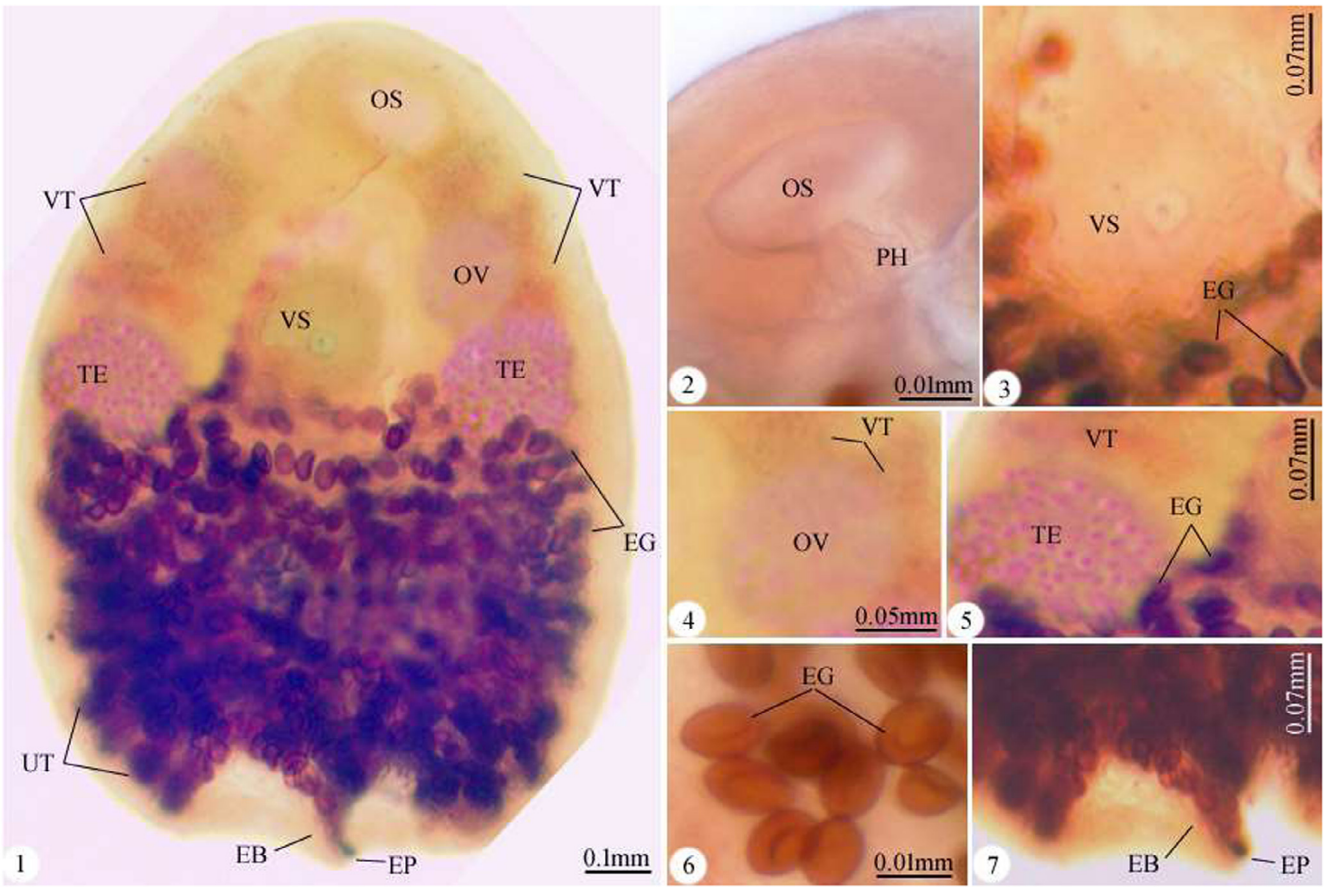
3. Results

The small intestine of the adult specimens Marsh frog *Rana Ridibunda* (F: Ranidae) were found to be parasitized by *Pleurogenoides medians* (F: Lecithodendriidae) reaching a prevalence rate of 63.33% (190/300). The highest percentage of infection was recorded in winter reaching 93.33% (70 specimens out of 75). The rate decreased gradually to 80% (60 out of 75) and 53.33% (40 out of 75) in spring and autumn, respectively. The lowest value of infection was detected in summer when only 6.66% (20 out of 75) fish were infected. Prevalence and mean intensity of infection were negatively correlated with the host size as smaller frogs (35 to 57 mm in length and <79 gm in weight) are more infected than larger ones (75–95 mm in length and >80 gm in weight). The numbers of parasites of male and female *R. ridibunda* were compared, and no significant differences were observed.

Microscopic examination (Figs. 1–17) The body is small, oval, measured 1.980–2.430 (2.205 ± 0.1) mm by 0.870–1.20 (9.87 ± 0.01) mm in size. The tegument is carpeted with regularly arranged flattened hand-like spines and each spine is composed of 5–8 finger-like appendages. Spines more densely arranged toward the anterior end and more sparsely distributed toward the posterior end, although spines distributed all over the body surface were similar in size. The oral sucker was sub-terminal, measured 0.180–0.230 (0.203 ± 0.01) mm long and 0.120–0.180 (0.150 ± 0.01) mm wide; the ventral sucker was smaller than the oral sucker, post-ovarian located at 1/3 level from the anterior end, measured 0.080–0.102 (0.090 ± 0.001) mm long and 0.100–0140 (0.120 ± 0.001) mm wide. The muscular pharynx was globular, measured 0.080–0.0120 (0.010 ± 0.001) mm long and 0.019–0.026 (0.021 ± 0.001) mm wide and followed by a short esophagus measured 0.075–0.089 (0.082 ± 0.001) mm long and 0.082–0.095 (0.090 ± 0.001) mm wide which bifurcated into two short ceca terminated at the level of the ventral sucker. Two large ovoid and symmetrical testes located laterally at the left median third part of the body near the cecal termination on both sides of the ventral sucker, measured 0.153–0.193 (0.176 ± 0.01) mm long and 0.160–0.192 (0.175 ± 0.01) mm wide. The ovary was sub-median to the left testis, pre-acetabular and measured 0.130–0.150 (0.140 ± 0.01) mm long and 0.100–0.130 (0.120 ± 0.01) mm wide. Uterus was long, coiled, filled with eggs, occupy the posterior half of the body, and narrowed at the level of the ventral sucker to form a metraterm. Seminal vesicle was tubular, winding, prostate complex well-developed. The cirrus pouch was club-shaped, situated on the left side in front of the ventral sucker and measured 0.350–0.460 (0.390 ± 0.01) mm long and 0.150–0.180 (0.170 ± 0.01) mm wide. Genital atrium was large, rounded enclosing a prominent genital pore which is situated on the left side of the oral sucker. Vitellaria are extra-caecal and extend from the level of the pharynx to a level slightly beyond the ovary. Eggs were small, oval, and operculated, amber colored and measured 0.032–0.041 (0.039 ± 0.001) mm long and 0.012–0.015 (0.014 ± 0.001) mm wide.

3.2. Taxonomic summary

Parasite: *Pleurogenoides medians* Olsson, 1876 belonging to Family Lecithodendriidae Odhner, 1911

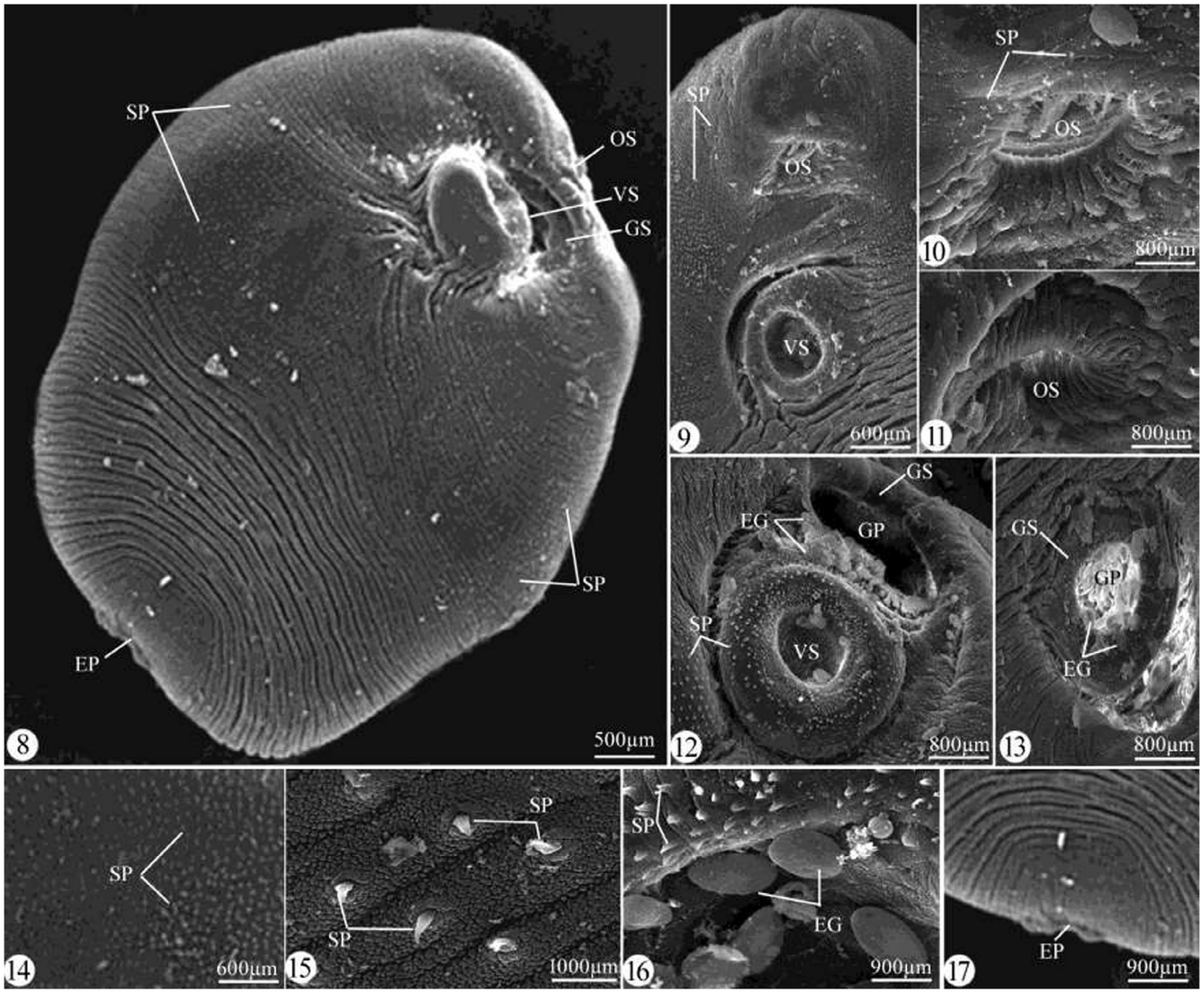


Figs. 1-7. Photomicrographs of *P. medians* showing: 1 Adult worm with oral sucker (OS), ventral sucker (VS), ovary (OV), testes (TE), uterus (UT) filled with eggs (EG), vitellaria (VT), body covered with tegumental spines (SP) and ended by excretory bladder (EB) opened by excretory pore (EP). 2 Oral sucker (OS) followed by pharynx (PH). 3 Ventral sucker (VS). 4 Ovary (OV). 5 Testes (TE). 6 Eggs (EG). 7 Excretory bladder (EB) opened to the outside by excretory pore (EP).

Type host: Marsh frog *Rana Ridibunda* Pallas, 1771 belonging to Family Ranidae Rafinesque, 1814
Site of infection: Small intestine of the infected frog specimens
Host Locality: El-Giza province, Egypt
Prevalence of infection: 63.33% (190 out of 300 were infected)
Material deposition: Voucher specimens deposited in the Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

4. Discussion

The marsh frog *R. ridibunda* was the largest frog native to Europe and belonged to the family of true frogs (Darrel, 2013). In fact to date, helminth parasites occurring in the Marsh frog were poorly known (Yildirimhan et al., 2005). The present study was planned to increase our knowledge about the helminthic fauna infecting the marsh frog by investigating one of the digenean parasites infecting frog specimens collected from El-Giza province, Egypt. To fulfill the aim of this study, a total of 300 frog specimens were collected from the studied area and examined for parasitic infections. The present parasitological examination revealed that percentage of the prevalence of parasitization of digenean parasites in the examined frog specimens was 63.33% (190/300); these are coincided with Abu Ahmed and Begum (2006) who stated that the total infestation rate was 69.23% for the presence of *Pleurogenoides petropedatis* in *Bufo melanostictus* collected from the Sonagazi area of Feni district, Bangladesh. In addition, the present study recorded that the prevalence of infection reached its maximum value during winter and lowest value of infection was detected in summer, which coincided with Kirin and Buchvarov (2002) followed by McAllister and Bursey (2004) and Yildirimhan et al. (2006) whom reported that the level of parasitic infections reached to the maximum levels during summer with very rarely values during autumn and winter as all amphibians undergoes hibernations during these seasons. Our findings showed that frogs of smaller size are more infected than larger ones and host sex showed no effect in this respect, which agreed with the hypothesis of Saglam and Arikan (2006) who stated the presence of a direct consequence of variable host behavior and immunity with age and size reflect on the level of parasitic infections. In addition, infections of trematodes, nematodes and acanthocephalans did not differ significantly between male and female *R. ridibunda* at 5%, indicating no effect of host sex on the diversity of endohelminthic fauna. *Pleurogenoides* was a well-known and common genus of intestinal flukes infecting frogs in the Palaearctic (Andreas, 2006). Travassos (1921) characterized genus *Pleurogenoides* by a body covered with spined tegument; oral and ventral suckers were almost of the same size; a small, round and muscular pharynx right next to the oral sucker followed by a short esophagus; and the branches of the intestine were short, ending at the same level as the ventral sucker; the well-developed cirrus pouch was opening to outside from the side; vitelline follicles were located on either side at the level of the pharynx and esophagus; ovary was situated among the intestinal pouches; testes located above the intestinal pouches; and excretory pouch of Y-shaped. Since the morphology of the present parasite species coincided with these features, it should be allocated within the species of the genus *Pleurogenoides* belonging to the Family Lecithodendriidae of the Phylum Trematoda. The digenean



Figs. 8-17. Scanning electron micrographs of *P. medians* showing: 8 Adult worm with oral sucker (OS), ventral sucker (VS), genital sucker (GS), body covered with tegumental spines (SP) and ended by excretory pore (EP). 9–17 High magnifications of: 9 Anterior part with oral sucker (OS) and ventral sucker (VS). 10,11 Oral sucker (OS). 12 Ventral sucker (VS), genital sucker (GS) opened outside by genital pore (GP) and filled with eggs (EG). 13 Genital sucker (GS). 14,15 Part of the body surface covered with tegumental spines (SP). 16 Eggs (EG). 17 Excretory pore (EP).

trematode belonging to *Pleurogenoides* found in the present study resembled and corresponded morphologically to *P. medians* described previously by Olsson (1876), Mathias (1924), Neuhaus (1941), Vojtkova (1974) and Smyth and Smyth (1980) as shown in (Table 1). The present form, however, differs from those described by these authors as well as from the rest known species of *Pleurogenoides* in having: (i) highly increased tegumental surface with densely arranged spines to be active in absorption of nutrients and digestion of host cells, (ii) an ovary which is situated to the left of the ventral sucker, (iii) a genital pore which is present near the right body margin at the level of pharynx. In addition, it differs from all other comparable species in the geographical distribution of their hosts except *P. compactus* and *P. stromi* having the same host; from *P. tener*, *P. spaericus*, *P. taylori*, *P. stromi*, *P. japonicas*, *P. compactus* and *P. amini* in having smaller body size; from *P. tener*, *P. spaericus*, *P. stromi* and *P. japonicas* in having smaller oral and ventral sucker; *P. taylori* and *P. compactus* in having smaller oral and larger ventral sucker; from *P. amini* in having larger oral and ventral sucker, from *P. japonicas* in extending the blind intestinal caeca above the level of the testicles; from *P. spaericus*, *P. taylori* and *P. compactus* by having unequal and smaller testes; from *P. tener, P. spaericus, P. japonicas* and *P. amini* by having a more compact uterus filled with smaller eggs size; from *P. amini* and *P. compactus* by having long caeca and more numerous vitelline follicles. The differences noted above appeared to be sufficient to treat the present parasite species as a new variety of *P. medians*. The geographic range for frog infection with *P. medians* has been reported worldwide and this trematode was the most common encountered frog intestinal parasite in Europe, Asia and Australasian Regions (Dawes, 1963; Cox, 1971; Hristovski and Lees, 1973; Brooks, 1976; Prudhoe and Bray, 1982; Gupta and Chopra, 1985). It has been reported in various amphibians and reptiles species, *Bufo vulgaris* (Yamaguti, 1958), *Lacerta trilineata* (Yamaguti, 1963; Yildirimhan, 1999), *B. bombina* (Vojtkova and Vojtek, 1975), *B. calamita* (Vojtkova and Vojtek, 1975), *R. arvalis* (Vojtkova and Vojtek, 1975), *R. esculenta* (Vojtkova and Vojtek, 1975; Buchvarov, 1977; Kuc and Sulgostowska, 1988b), *R. temporaria* (Vojtkova and Vojtek, 1975; Cedhagen, 1988), *H. arborea* (Vojtkova and Vojtek, 1975; Düşen and Öz, 2004), *B. variegata* (Vojtkova and Vojtek, 1975; Shimalov and Shimalov, 2001), *Triturus vulgaris* (Vojtkova

Table 1
Comparative measurements (in millimeters) of the present *P. medians* and those described previously.

Related species	*Pleurogenoides medians*	*P. tener*	*P. spaericus*	*P. taylori*	*P. stromi*	*P. japonicus*	*P. compactus*	*P. amini*	*P. medians*
Parameters	Olsson (1876)	Looss (1898)	Klein (1905)	Tubangui (1928)	Travassos (1930)	Yamaguti (1936)	Strohm (1940)	Biigees et al. (2005)	(Present study)
Host	*Bufo bufo*	*R. mascereniensis*	*Rana* sp.	*Rana limnocharis*	*Rana ridibunda*	Dragonflies	*Rana ridibunda*	*Rana tigrina*	*Rana ridibunda*
Locality	Valencia, Spain	United Arab Republic, Tunisia	Vicinity of Chiang Mai	Luzon Island, Philippines	Saudi Arabia	Chungnam, Chonbuk provinces	Saudi Arabia	Karachi University Campus, Pakistan	El-Giza province, Egypt
Site of infection	Small intestine	Small intestine	Intestine	Stomach, small intestine	Intestine	Small intestine	Small intestine	Small intestine	Small intestine
Body length	2.63	0.79–1.3	0.43–1.12	0.980	0.46–0.84	1.80–2.20	1.30–1.59	1.09	1.980–2.430 (2.205 ± 0.1)
Body width	0.97	0.64–1.03	0.32–0.83	0.501	0.37–0.77	0.70–0.91	0.590–0.690	0.73	0.870–1.20 (9.87 ± 0.01)
Oral sucker length	–	0.15–0.17	0.093–0.022	0.133	0.08–0.14	0.18–0.24	0.147	0.22	0.180–0.230 (0.203 ± 0.01)
Oral sucker width	0.16	–	0.099–0.25	0.155	0.11–0.21	–	0.176–0.139	0.19	0.120–0.180 (0.150 ± 0.01)
Ventral sucker length	–	0.17–0.20	0.091–0.198	0.136	0.09–0.19	0.17–0.23	0.133–0.183	0.13	0.080–0.102 (0.090 ± 0.001)
Ventral sucker width	0.13	–	0.038–0.198	0.143	0.11–0.18	–	0.191	–	0.100–0140 (0.120 ± 0.001)
Pharynx length	–	0.05–0.07	0.018–0.098	0.076	–	0.05–0.07	0.063	0.045	0.080–0.0120 (0.010 ± 0.001)
Pharynx width	–	–	0.021–0.097	0.057	0.03–0.06	–	0.069	0.057	0.019–0.026 (0.021 ± 0.001)
Esophagus length	0.078	0.12	–	–	0.05	–	0.200	0.030	0.075–0.089 (0.082 ± 0.001)
Esophagus width	–	–	–	–	–	–	–	0.042	0.082–0.095 (0.090 ± 0.001)
Right Testis length	–	0.18–0.36	0.182–0.295	0.157	0.12–0.30	0.25–0.34	0.176–0.132	0.12	0.153–0.193 (0.176 ± 0.01)
Right Testis width	0.19	0.12–0.16	0.131–0.237	0.172	0.09–0.18	–	–	0.15	0.160–0.192 (0.175 ± 0.01)
Left Testis length	–	0.18–0.36	0.148–0.270	0.184	0.12–0.30	0.25–0.34	0.160–0.130	0.15	0.153–0.193 (0.176 ± 0.01)
Left Testis width	0.19	0.12–0.16	0.128–0.237	0.111	0.09–0.18	–	–	0.10	0.160–0.192 (0.175 ± 0.01)
Ovary length	–	0.17	0.068–0.154	0.133	–	0.24–0.29	0.120	0.12	0.130–0.150 (0.140 ± 0.01)
Ovary width	0.125	0.13	0.048–0.176	0.099	0.08–0.17		0.100	0.13	0.100–0.130 (0.120 ± 0.01)
Cirrus pouch length	–	–	–	0.345	0.12–0.28	0.433–0.69	0.470–0.650	0.37	0.350–0.460 (0.390 ± 0.01)
Cirrus pouch width	–	–	–	0.159	0.08–0.12	–	0.110–0.147	–	0.150–0.180 (0.170 ± 0.01)
Eggs length	0.036	0.026–0.031	0.021–0.032	0.030–0.032	0.017–0.025	–	0.022–0.027	0.020–0.028	0.032–0.041 (0.039 ± 0.001)
Eggs width	0.015	0.013–0.017	0.013–0.017	0.015–0.018	0.01–0.014	–	0.011–0.012	0.010–0.011	0.012–0.015 (0.014 ± 0.001)

and Vojtek, 1975; Shimalov et al., 2001), *R. ridibunda* (Buchvarov, 1977; Kuc and Sulgostowska, 1988a; Sattmann, 1990; Oğuz et al., 1994; Yildirimhan et al., 1996; Mashaii et al., 2000; Yildirimhan et al., 2005; Düşen and Öz, 2006; Saglam and Arikan, 2006), *R. dalmatina* (Buchvarov 1977; Düşen et al. 2009), *B. bufo* (Shimalov and Shimalov, 2000, 2001), *L. agilis* (Sharpilo et al., 2001), *T. cristatus* (Shimalov et al., 2001), *Natrix natrix* (Kirin, 2002a), *R. holtzi* (Topçu, 2002), *R. camerani* (Yildirimhan et al., 2006; Düşen, 2007), *R. macrocnemis* (Yildirimhan et al., 2006; Düşen, 2007), *B. viridis* (Düşen et al. 2010; Düşen and Oğuz, 2010), *Atheris hispida* (Hassl, 2010; Hassl et al., 2010), *H. savignyi* (Yildirimhan et al., 2012), and *Pelophylax ridibundus*(Düşen and Öz, 2013).

Therefore, it could be concluded that the present study supplied valuable information on the occurrence and prevalence of the recovered digenean parasite *P. medians* for the first time infecting *R. ridibunda* in Egypt. In addition, future studies are recommended to include explanation about the role of the tegumental surface area in *Pleurogenoides* nutrition and advanced molecular characteristics for this species.

Conflict of interest

Authors stated that there is no conflict of interest.

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