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New host and locality record for the digenean parasite, *Paracephalogonimus minutus* Mehra, 1937 (Digenea: Cephalogonimidae) in *Hoplobatrachus tigerinus* of YSR Kadapa District, Andhra Pradesh, India

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ABSTRACT

During an inventory of helminth parasites of amphibians from several localities of YSR Kadapa district, Andhra Pradesh, a digenean parasite, *Paracephalogonimus minutus* Mehra, 1937 was recovered from the intestine of the host, *Hoplobatrachus tigerinus* of YSR Kadapa District, Andhra Pradesh. This parasite is reported exclusively from turtles and in the present study it is first recorded in *Hoplobatrachus tigerinus* from YSR Kadapa region and hence, new host and locality record for the parasite was established. The morphological and diagnostic characteristics of *Paracephalogonimus minutus* Mehra, 1937 was studied by means of light microscopy and scanning electron microscopy.

Keywords: Digenea, *Paracephalogonimus minutus*, *Hoplobatrachus tigerinus*, YSR Kadapa.

1. INTRODUCTION

Amphibian hosts, like other hosts serve as the most preferred vertebrate hosts for a number of metazoan endoparasites like digeneans, cestodes, nematodes, acanthocephalans and protozoans and ectoparasites such as monogeneans and leeches [1,2]. Their normal growth is hindered by the presence of heavily infected endoparasites [3]. There are several areas in India, in particular in Southern India especially YSR Kadapa where the biodiversity of metazoan parasites in vertebrate hosts are poorly investigated. Anuran frogs and toads are a group of vertebrate hosts abundantly found in the warm and humid climates of YSR Kadapa District, Andhra Pradesh. Hoplobatrachus tigerinus Daudin, 1803 (Dicroglossidae Anderson, 1871) was the most prevalent species of amphibians of YSR Kadapa District, i.e., repeatedly occurring aquatic species of anurans dwelling in the low lying areas like ponds, ditches and irrigated fields. Trematodes otherwise known as flukes or digeneans may be the most

widely recognized of the amphibian parasites. Amphibians may serve as a secondary intermediate or definitive final host for trematodes and most the works contributed by eminent scientists all over the world was on the community structure of the parasites [4-31]. Scanning electron microscopy (SEM) provides a wonderful means to define the accurate surface topographical features of a species than light microscopy [32]. There are very few reports on the surface topography of the digentic trematodes from various vertebrate hosts [33-45]. The high magnification and 3-D photographs acquired with the SEM facilitates us to know the spatial relationship of surface structures in detail to evaluate them as taxonomic characters [46]. Cephalogonimids are the digenetic trematodes found in amphibians and reptiles.

The genus *Paracephalogonimus* belonging to the sub-family Cephalogoniminae of the family Cephalogonimidae Nicoll (1914) was first established by Mehra (1937) and later by Skrjabin (1950) with *Paracephalogonimus minutus* as its type-species from Lissemys punctatus [47-49]. The parasites of this genus are most common parasites of chelonians. But, in the present study, it is reported in huge numbers from the intestine and rectum part of the amphibian host, *Hoplobatrachus tigernius* of YSR Kadapa district, Andhra Pradesh. The present aim of the study is to describe the digenean parasite, *Paracephalogonimus minutus* for the first time from *Hoplobatrachus tigerinus* in YSR Kadapa District, Andhra Pradesh 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 Andhra Pradesh. Hence, *Hoplobatrachus tigerinus* serve as new host record and YSR Kadapa as new locality record for the parasite.

2. MATERIALS AND METHODS

2.1. Sample collection and Parasitological examination

Three hundred adult frogs, *Hoplobatrachus tigerinus* were collected with the aid of butterfly nets or by hand from four different sites of YSR Kadapa District (Lat. 14°28´N 78°49´E, 137 m Altitude), located in Andhra Pradesh state during February, 2013 to February, 2015. The collection sites include:

Site-1: Industrial Estate area (Lat. 14°47´N 78°76´E, 138 meters Altitude), YSR Kadapa district,

Site-2: Campus area of Yogi Vemana University (Lat.14°28´N 78°49´E, 137 m Altitude), located in YSR Kadapa District of Andhra Pradesh,

Site-3: Ramapuram village (14.05°N 78.75°E, 143 meters) Raychoti Mandal,

Site-4: Bouinpalli village, Kadapa (Lat.14º28'N 78º52'E, 379 meters).

Host samples of different sizes i.e., small, medium and large were transported to laboratory and were instantly scrutinized after being euthanized. The various parts of the gastro intestinal tract-oesophagus, stomach, intestine and rectum was segregated and each part of which was split and its contents were scraped into a petridish containing 0.7% solution or Ringer's solution. These tissues are teased out with needles and examined for the presence of helminthes under a stereozoom microscope (LM-52-3621 Elegant). These helminth parasites were found attached to intestine of the host. Collected specimens were temporarily preserved in A.F.A (Alcohol-85%, Formalin- 5%, Glacial acetic acid- 10%) for 24 hours which acts as an ideal fixative for the whole mount preparations and stained with alum caramine. The specimens after being in alum caramine washed thoroughly and dehydrated by using a graded sequence of ethanols (70%, 80%, 95%, 100%) cleared in xylene and finally mounted in Canada balsam or DPX mount on a glass slide with coverslip and proper care is taken to avoid formation of any air bubbles during mounting. The conventional techniques for the preparation of permanent slides were adopted from Hiware *et al*., (2003) and Madhavi *et al.*, (2007) [50, 51]. The stained parasites were observed and identified under the Lynx trinocular microscope (N-800M) and their microphotographs were captured and line diagrams were drawn with the aid of attached drawing tube. For SEM specimens, the parasites were fixed in 2.5% glutarldehyde in 0.1 M phosphate buffer (pH 7.2) at 4˚C for 1 hour. They were washed in the same buffer before post-fixation in 1% osmium tetroxide in the same buffer at 4˚C for 1 hour. Specimens were dehydrated through graded series of ethanol (70%-100% at 5-10 min interval), critical point dried and sputter coated with gold. SEM photographs at various magnifications were captured with a Carl Zeiss Scanning electron microscope (ΣIGMA TM) facility at Sri Venkateswara University, Tirupati. Identifications were made according to Yamaguti (1958) [52].

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 IAEC (Institutional Animal Ethics Committee) – Registration Number 1460/PO/a/11/CPCSEA, dated 20.05.2011), Zoology Department in Faculty of Life Sciences, Yogi Vemana University, Andhra Pradesh.

3. RESULTS

The small intestine and rectum of the adult specimens of *H. tigerinus* were found to be parasitized by *Paracephalogonimus minutus* (Digenea: Cephalogonimidae) reaching a prevalence rate of 13.67% (41/300). The highest percentage of infection was recorded in winter reaching 17% (16/94). The rate decreased gradually to 13.4% (13/97) in summer and lowest in 11.3% (12/106) in rainy season. A total of 5009 P. minutus (range=1-1200) were recovered from these infected hosts. The medium sized frogs (12 to16 cm in length and 178gm in weight) are more infected than larger ones (16-20 cm in length and 265gm in weight). The weak correlation coefficient, r=0.039 shows a weak correlation between host snout length and parasitic abundance. The number of parasites of males and females *H.tigerinus* were compared (Mann-Whitney U-test, Z-score=-0.79, p=0.423 at p<0.05) and no significant differences were observed.

3.1 Microscopic examination

(Plate-1; as shown in Figure. 1, 2 & 3)

The body is very small, nearly rounded, spinose and measure upto 0.62-0.78 × 0.33-0.57 mm in size. Forebody measures 0.23-0.39 × 0.5-0.47 mm in size. Oral sucker subterminal, larger than acetabulum and measures 0.05-0.1 × 0.05-0.1mm. Hind body measures upto 0.23-0.4 × 0.32-0.54 mm in size. Prepharynx present, pharynx well developed but small and measures 0.02-0.05 × 0.03-0.05 mm. Oesophagus very small or sometimes practically absent. Caeca short and terminating a little behind posterior testis and measures 0.06-0.1 mm. Acetabulum in mid region of body measures 0.03-0.06 × 0.02-0.04 mm. Testis diagonal, postacetabular, anterior testis measures 0.3-0.32 × 0.2-0.45 mm. Posterior testis measures 0.29-0.31 × 0.18-0.35 mm. Cirrus pouch is big and claviform, extending to ovary passing immediately in front of acetabulum containing large sac like structure 0.20-0.25 × 0.05-0.09 mm. Ovary oval dextral to acetabulum at equatorial level and measures 0.04-0.11 × 0.04-0.1 mm. Vitelline follicles present in lateral regions on the right side 0.020-0.034 × 0.018-0.0763 mm and in the left side 0.0275-0.0520 × 0.0225-0.0789 mm. Uterus occupying most of body posterior to vitellaria. Eggs small measures 0.013-0.028 × 0.010-0.028 mm (Plate-1; Figure. 1 & 2).

3.2 SEM examination

Body is oval in shape, minute with mouth being terminal in position, followed by pharynx. Oral sucker is present in the anterior region and ventral sucker in the middle half of the body. The tegumental surface is carpeted with uniformly arranged small spines all over the body from anterior region to posterior region (Plate-1; Fig. 3). Ventral sucker is clearly visible. It appears that the spines are more densely arranged in uniform concentric rings towards posterior side than the anterior side, although the spines all over the body appear to be of similar size. Ovary oval in shape and dextral to ventral sucker (acetabulum). Testes diagonal and postacetabular in position.

3.1 Taxonomic summary

Parasite: *Paracephalogonimus minutus Mehra, 1937 belonging to family Cephalogonimidae Loss, 1899*

Type host: *Indian bull frog Hoplobatrachus tigerinus belonging to Dicroglossidae family*

Site of infection: *Intestine and rectum*

Host Locality: *YSR Kadapa District (Lat. 14°28´N 78°49´E, 137 m Altitude), located in Andhra Pradesh state.*

Prevalence of infection: *13.67% (41 out of 300 infected)*

Material deposition: *Voucher specimens deposited in the Department of Zoology, Faculty of Life sciences, Yogi Vemana University, YSR Kadapa, Andhra Pradesh, India.*

4. DISCUSSION

The Indian bull frog *Hoplobatrachus tigerinus* is the largest frog native to Asia and reaches upto 15cm in length. To date, helminth parasites occurring in Indian bull frog were poorly investigated from Southern India. The present study was planned to enhance our knowledge about the helminthic fauna infecting bull frog by investigating one of the digenean parasites infecting frog specimens collected from YSR Kadapa District, Andhra Pradesh. To accomplish this study, a total of 300 frog specimens were collected from the studied area and scrutinized for the parasitic infections. The present parasitological examination revealed that percentage of the prevalence of parasitization of digenean parasites in the examined frog specimen was only

Table-1: Comparative measurements (in millimeters) of the *P. minutus* with previously described species

Related species	***P.minutus* (Mehra, 1937) Skrjabin, 1950**	***P.hydrabadensis* Simha and Rao, 1969**	***P.osmaniae* Simha and Rao, 1969**	***Present P. minutus***
Parameters				
Host	*Lissemys punctata*	*Kachuga kachuga*	*Nilssonia (Trionyx) leithi*	*Hoplobatachus tigerinus*
Locality	India	Hyderabad (India)	Gulburga-India	YSR Kadapa, AP, India
Site of infection	Small intestine	Small intestine	Small intestine	Small intestine and rectum
Body length	0.52-0.63	-	-	0.62-0.78
Body width	0.38-0.49	-	-	0.33-0.57
Oral sucker length	-	Oral sucker larger than acetabulum	-	0.05-0.1
Oral sucker width	-	-	-	0.05-0.1
Acetabulum length	-	-	-	0.03-0.06
Acetabulum width	-	-	-	0.02-0.04
Pharynx length	-	-	-	0.02-0.05
Pharynx width	-	-	-	0.03-0.05
Anterior testis length	-	-	-	0.3-0.32
Anterior testis width	-	-	-	0.2-0.45
Posterior testis length	-	-	-	0.29-0.31
Posterior testis width	-	-	-	0.18-0.35
Ovary length	-	-	-	0.0-0.11
Ovary width	-	-	-	0.04-0.1
Cirrus pouch length	-	-	-	0.20-0.25
Cirrus pouch width	-	-	-	0.05-0.09
Eggs length	-	-	-	0.013-0.028
Eggs width	-	-	-	0.010-0.028

Plate-1

Fig. 1

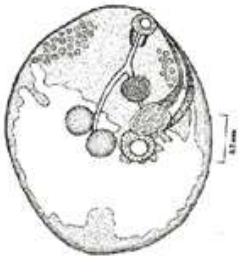

Fig. 2

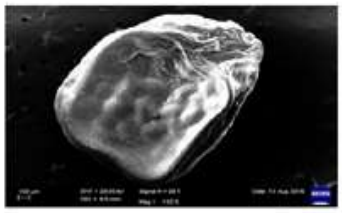

Fig. 3

Plate.1: Figure.1. Microphotograph of *Paracephalogonimus minutus* 100X; Figure.2 Line diagram 100X; Figure.3. SEM Photograph of *Paracephalogonimus minutus* 100µm.

13.67% (41 out of 300 infected) which shows that parasitization was very heavy in the infected frogs. In addition, the present study recorded that the prevalence of infection was maximum in winter followed by summer and least in Rainy season, which coincided with the studies of Kirin and Buchavarov (2002), Mc Allister and Bursey (2004) and Gaber et al., (2017) [53,54,45]. Our finding showed that the frogs of smaller and medium size are more infected than the larger ones and host sex showed no effect in this respect, which was in total conformity with the views of Saglam and Arikan (2006) who proposed that the presence of a direct outcome of variable host behavior and immunity with age and size reflect on the level of parasitic infections [55]. *Paracephalogonimus* was a well-known and common genus of intestinal and rectal flukes infecting chelonians [56]. It belonged to the sub-family *Cephalogoniminae Looss* (1899), which subsequently rose to family *Cephalogonimidae Nicoll* (1914) including four genera: *Cephalogonimus Poirier*, 1886, *Emoleptalea Looss*, 1900 (Syn: Leptalea Looss, 1899), *Paracephalogonimus Skrjabin*, 1950 and Oudhia Dayal and Gupta, 1954. The genus *Paracephalogonimus* was first erected by Mehra (1937) and later by Skrjabin (1950) with *Paracephalogonimus minutus* as its type-species from *Lissemys punctatus* [48, 49]. Simha and Rao (1969) reported two new species, *Paracephalogonimus hydrabadensis* from the intestine of turtle, *Kachuga kachuga* at Hyderabad (India) and *Paracephalogonimus osmaniae* from the intestine of *Nilssonia* (Trionyx) leithi at Gulburga (India) [56]. The digenetic trematode belonging to *Paracephalogonimus* found in the present study resembled and corresponded morphologically to *P. minutus* described previously from the chelonian host by Simha and Rao (1969) as shown in Table-1. The present form however differs in the host, position of vitellaria and slightly in the measurements. Therefore it can be concluded that the present study supplied valuable information that *Hoplobatrachus tigerinus* serve as a new host record and the YSR Kadapa district serve as new locality record for the parasite.

5. CONCLUSION

Some parasites are host-specific while others are not host-specific. *Paracephalogonimus minutus* is described as host-specific parasite exclusively found in chelonians such as turtles. But, in the present study, these parasites were recorded from the amphibian hosts of YSR Kadapa district for the first time. Hence, *Hoplobatrachus tigerinus* serve as new host record and YSR Kadapa District as a new locality record for the parasite.

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Conflicts of Interest

There are no conflicts of interest.

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