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ORIGINAL ARTICLE

FIRST REPORT OF *Plagiorchis vespertilionis* (Müller, 1780), A KNOWN ZOONOTIC FLUKE, WITH NOTES ON TWO SPECIES OF *Paralecithodendrium* (PLATYHELMINTHES: TREMATODA) FROM *Myotis* sp. AND *Miniopterus* sp. (MAMMALIA: CHIROPTERA) IN THE PHILIPPINES

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ABSTRACT

***Plagiorchis vespertilionis* (Müller, 1780) from the mouse-eared bat (*Myotis* sp.) is described and illustrated based on materials from the Philippines. It is reported for the first time in the country constituting a new locality record for the species. It is differentiated from other species of *Plagiorchis* declared in the country and also from species from bats in other countries. A pictorial comparison of the general morphology of *P. vespertilionis, P. philippinensis, P. potamonides* and *P. maculosus* is provided. Two other species but of the genus *Paralecithodendrium* namely *P. longiforme* (Bhalerao, 1926) from the mouse-eared bat and *P. ovimagnosum* (Bhalerao, 1926) and the bent-winged bat (*Miniopterus* sp.) are also reported, described, and illustrated based on the present specimens. *P. vespertilionis* and *P. longiforme* occurred as concurrent infection in the same host. This is the third account of the two species since these were first recorded in 1928 and last reported in1986.**

Keywords: *bat, Philippines*, *Paralecithodendrium* spp. *Plagiorchis vespertilionis, trematode, zoonotic parasite*

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INTRODUCTION

Bats belong to the mammalian order Chiroptera and are worldwide in distribution except in the north and south polar regions and a few oceanic islands (Mildenstein & de Jong, 2011). They play an important role in the maintenance of the ecosystem and agriculture such as seed dispersion, in pollination as well as controlling insect pest populations (FAO, 2011). They have, however, been implicated as important reservoir hosts for several pathogenic viruses that can cross species barriers to infect humans and other domestic and wild mammals (Calisher *et al.*, 2006). They are also known to host a variety of parasitic trematodes (Bray *et al.,* 2008) some of which are zoonotic.

The bat fauna of the Philippines consists of 79 species contained in seven families. The local bat fauna is particularly important due to the high levels of species richness and endemism coupled by the rapid rate of loss of their natural habitat (Heaney *et al*. 2010). The ectoparasites of bats in the Philippines appear to be well known as shown by the number of published works on this subject (Kohls, 1950; Brown, 1997; Bochov & OConnor, 2006; Zabat & Eduardo, 2011; Alvarez *et al*., 2016; Amarga *et al*, 2017). In contrast, published works on endoparasites are meager, particularly the parasitic trematodes. Only two works (Tubangui, 1928; Kifune & Sawada, 1986) that dealt on the trematodes of bats among other hosts in the Philippines have appeared in literature. Opportunities to examine bats for internal parasites (trematodes, cestodes, nematodes and acanthocephalan) are very few and far in between because of the need to open the animal to recover the internal parasites. Ectoparasites are easier to collect even from live animals with proper handling after which these are released. Therefore, any opportunity presented to examine bats for endoparasites at necropsy should not be missed or overlooked. This paper presents the author's observations on three species of trematodes from Philippine bats, one of which is already known to infect humans in other countries and is here reported for the first time; the other

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two species are herein reported after three decades from their last account in the country.

MATERIALS & METHODS

Specimens of bat trematodes examined in this study came from two sources and were received already preserved in 70 % alcohol and were therefore dead biological materials. Some specimens were collected and submitted in June 2016 from a bat (*Myotis* sp.) from Candaba Swamps (15°5'35.9988" N and 120°49'41.9916" E) in Pampanga, Luzon and additional trematode specimens from another bat (*Miniopterus* sp.) collected earlier in September 2015 but the specific locality origin in the Philippines was not indicated. Specimens were processed and examined in 2018 - 2019. Bats, as described by the collectors, were identified to the genus level using the work of Heaney *et al*. (2010) on Philippine mammals.

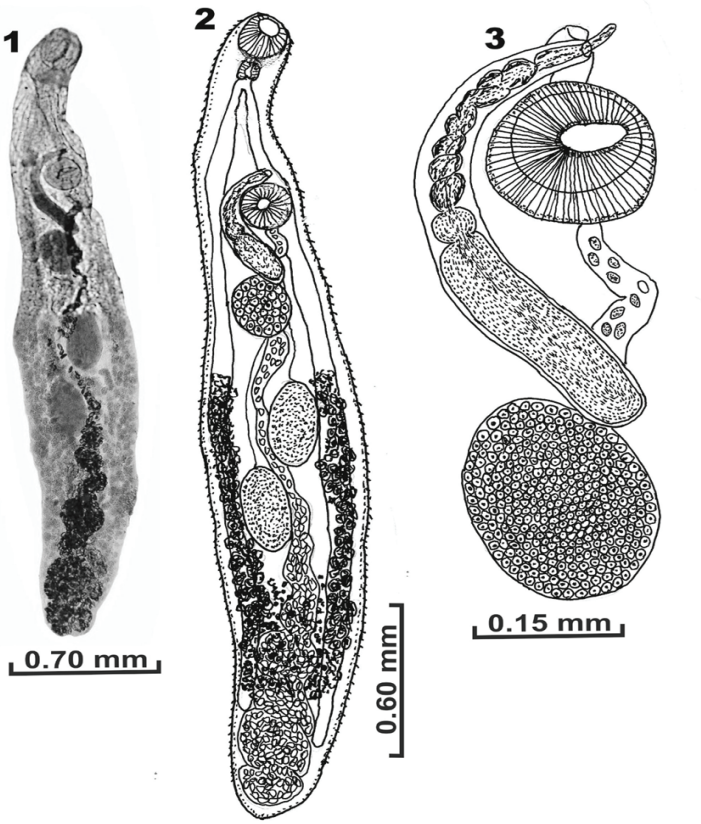
The trematodes were immediately transferred and stored in 80% ethyl alcohol until further study. Whole mounts were prepared by staining in borax-carmine overnight, de-stained in acid alcohol (1% HCl in 80% ethyl alcohol) until the desired pinkish color is reached, neutralized in 1% alkaline alcohol (1% sodium hydroxide in 80% ethyl alcohol) to arrest further staining, dehydrated in increasing grades of ethyl alcohol, cleared in terpineol, and mounted on glass slides in Canada balsam. In addition, Philippine specimens of the following species were also examined for comparative purposes: *Plagiorchis philippinensis*, *P. potamonides* from field rats (*Rattus tanezumi*) kept at the PCRC-CVM and *P. maculosus* from birds, a photograph of which was Measurements were made with the aid of a calibrated eyepiece micrometer attached to an Olympus CX31 research microscope (Olympus Optical Co. Ltd, 2-43-2, Hatagaya, Shibuya-ku, Tokyo, Japan). Photomicrographs were taken using a Nikon biological microscope Eclipse E2000 with digital sight capture attachment DS-Fil (Nikon Corporation, 916, Ohi 3-chome, Shinagawa -ku, Tokyo 140-8505, Japan). Drawings were made with the aid of a drawing apparatus attached to an Olympus CX31 research microscope (same address as above). Voucher specimens were deposited in the Parasite Collection and Reference Center of the College of Veterinary Medicine, University of the Philippines Los Baños.

RESULTS

After careful examination, the present materials of trematodes from bats consisted of three species namely: *Plagiorchis vespertilionis* (N=17) of the family Plagiorchiidae, superfamily Plagiorchioidea; the other two species are: *Paralecithodendrium longiforme* (N=1) and *P. ovimagnosum* (N=4) of the family Lecithodendriidae, superfamily Microphalloidea. *P. vespertilionis* and *P. longiforme* were found in the intestine of a mouse-eared bat (*Myotis* sp.) occurring as a concurrent infection while *P. ovimagnosum* from the intestine of a bent-winged bat (*Miniopterus* sp.). These are described, with accompanying microphotographs and drawings, and discussed below based on the present materials.

Phylum Platyhelminthes
Class Trematoda
Superfamily Plagiorchioidea
Family Plagiorchiidae
Genus *Plagiorchis* Lühe, 1901

Plagiorchis vespertilionis (Müller, 1780)
(Figs. 1-3)

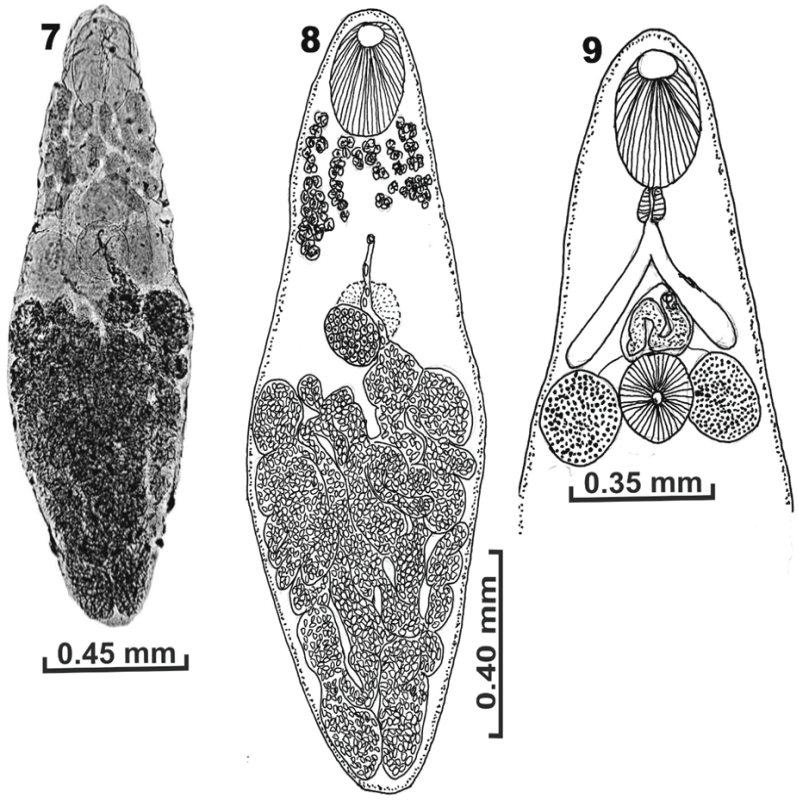


Figures. 1-3. *Plagiorchis vespertilionis*. Fig. 1. Whole worm, habitus form (aceto-alum carmine stain); 2. Whole worm drawn to a closer view showing all features; 3. Cirrus and the structures contained within in relation to the ovary, terminal part of uterus, and the acetabulum.

Figures 4-6. Other *Plagiorchis* spp. (Philippine materials). 4. *P. philippinensis* (from rat, *Rattus tanezumi*); 5. *P. potamonides* (from rat, *Rattus tanezumi*); 6. *P. maculosus* (from bird, *Artamus leuchorynchus*).

Body elongate, tegument with spines, measures 2.60-4.80 mm in length, and 0.40-0.58 mm in greatest width attained at level of testicular region. Oral sucker subterminal, measures 0.156-0.162 by 0.138-0.159 mm. Ventral sucker or acetabulum in anterior third of the body, measures 0.155-0.163 by 0.154-0.162 mm, nearly equal in size or slightly larger than oral sucker. Oral sucker/ventral sucker ratio, 1:1-1.2. Prepharynx absent; pharynx 0.001-0.004 by 0.001 -0.004 mm; esophagus very short or nearly absent; ceca run along lateral sides terminating blindly about 0.365-0.401mm from posterior end of the body. Testes oblong and smooth, slightly oblique one after the other in the anterior region of the posterior half of the body, anterior testis measures 0.314-0.320 by 0.085-0.092 mm, posterior testis measures 0.330-0.341 by 0.170-0.176 mm. Cirrus sac extending from genital pore anteriorly and curving along right side of acetabulum and terminate posteriorly reaching anterior border of ovary, measures 0.536=0.548 long, contains a bipartite seminal vesicle, pars prostatica and smooth cirrus; seminal receptacle absent. Ovary round, smooth posterior to cirrus sac and anterior to testes, measures 0.315-0.350 by 0.086-0.094 mm; Vitellaria in lateral fields, overlapping ceca, anterior limit at level of anterior testis and terminating at a distance about 0.626-0.635 mm from posterior end of body, confluent medially in their posterior limit; uterus filled with eggs, descending and ascending loops occupy median area from posterior end running towards anteriorly between testes as well as between anterior testis and ovary and terminating at the genital pore which is just anterior to the acetabulum; egg operculate, measures 29-35 by 15-21 microns.

Superfamily Microphalloidea
Family Lecithodendriidae
Genus *Paralecithodendrium* Travassos, 1921
(Figs. 4-6)



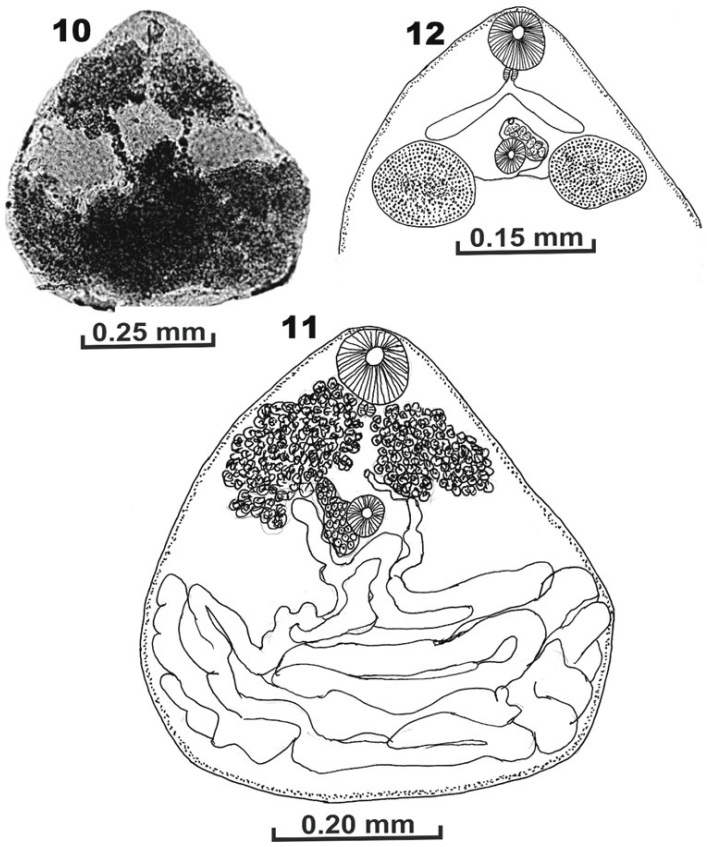
Figs. 7-9. *Paralecithodendrium longiforme*. Fig. 7. Whole worm, habitus form (aceto-alum carmine stain); 8. Whole worm but showing only the suckers and female reproductive organs; 9. Anterior half of worm showing only the suckers, digestive, and female reproductive organs.

Paralecithodendrium longiforme (Bhalerao, 1926)
(Syn.: *Lecithodendrium luzonicum* Tubangui, 1928)
(Figs. 7-9)

Body elongate, attenuated anteriorly and posteriorly, broader at the midbody, measures 1.720 mm in length and 0.650 mm in greatest width attained at the midbody region. Oral sucker, well-developed, cup-shaped, subterminal, measures 0.294 by0.207 mm. Ventral sucker or acetabulum in anterior border of the posterior half of the body, between the testes, smaller than oral sucker, measures 0.181 by 0.181 mm, ventral sucker/oral sucker ratio, 1:1.62. Prepharynx absent; pharynx measures 0.086 by 0.068 mm; esophagus absent; ceca very short terminating blindly anterior to testes. Testes nearly spherical and smooth, symmetrical in position in lateral area, in either side of acetabulum right testis measures 0.198 by 0.198 mm, left testis measures 0.193 by 0.162 mm. Cirrus sac prominent, nearly round, in median line just anterior to acetabulum, measures 0.206 mm long and 0.154 mm in width, contains a coiled seminal vesicle, pars prostatica and cirrus; seminal receptacle absent. Ovary round and smooth, dorsal to and overlapping posterior half acetabulum, measures 0.115 by 0.111 mm; Vitellaria in anterior half of the body, occupying area between oral sucker and testes and acetabulum, confluent medially, uterus form descending and ascending transverse loops, occupying whole area posterior to the testes, filledwith eggs, then running towards anteriorly and medially, then dorsal to acetabulum and terminating at the genital pore which is just posterior to the cecal bifurcation; egg measures 35-39 by 14-19 microns.

Paralecithodendrium ovimagnosum (Bhalerao, 1926)
(Figs. 10-12)

Body pyriform to oval, measures 0.410-0.552mm in length and 0.410-0.613 mm in greatest width attained at the level posterior to



Figs. 10-12. *Paralecithodendrium ovimagnosum*. 10. Whole worm, habitus form (aceto-alum carmine stain). 11. Whole worm but showing only the suckers and the female reproductive organs; 12. Anterior half of worm showing only the suckers, digestive organs, and the male reproductive organs.

the testes. Oral sucker subterminal, measures sucker or acetabulum in anterior half of the body, smaller than oral sucker, measures 0.041-0.044 by 0.042-0.044 mm. Ventral sucker/oral sucker ratio, 1:2. Prepharynx absent; pharynx small measures 0.012-0.014 by 0.013-0.015 mm; esophagus absent; ceca very short terminating blindly anterior to testes. Testes nearly spherical and smooth, symmetrical in position in lateral area posterior to the cecal ends with acetabulum in between at the midline, right testis measures 0.120-0.128 by 0.154-0.160 mm, left testis measures 0.095-0.098 by 0.142-0.149 mm. Cirrus sac prominent, oblong, anterior to acetabulum, measures 0.087-0.090 mm long, 0.032-0.036 mm wide, contains a coiled seminal vesicle, pars prostatica and non-protrusible cirrus; seminal receptacle absent. Ovary oblong and lobed immediately on the right side of the acetabulum measures 0.085-0.092 by 0.035-0.040 mm; Vitellaria in anterior half of the body, occupying area between oral sucker and testes, uterus form descending and ascending transverse loops, occupying whole area posterior to the testes, filled with eggs, then running towards anteriorly between testes and terminating at the genital pore which is just anterior to the acetabulum; egg measures 24-29 by 12-16 microns.

DISCUSSION

The present materials of *Plagiorchis* conform to the diagnosis of that genus in the key to the genera of the family Plagiorchiidae as provided by Tkach (2008) and essentially agree with the description of *Plagiorchis vespertilionis* by previous workers (Tkach *et al*., 2000; Kirilov *et* al., 2012; Guk *et al*., 2007). Members of the genus *Plagiorchis* are parasites of tetrapods (Tkach, 2008) and some species including *P. vespertilionis* have been recorded in both animals and humans, thus, are considered zoonotic parasites (Guk *et al*., 2007; Chai, 2019). In the Philippines, the following species of the genus *Plagiorchis* and their host have been reported: *Plagiorchis muris* from

rats, *P. philippinensis* from rats and humans, *P. potamonides* from rats (Eduardo & Lee, 2006; Eduardo *et al.*, 2008); *P. maculosus* from birds (Fischthal & Kuntz, 1972) and *P. dilimanensis* experimentally in laboratory mice (Velasquez, 1964). *Plagiorchis vespertilionis* differs from the above species except *P. maculosus* in the extent and distribution of the vitellaria. The vitellaria of unlobed ovary while that of the latter species, the ovary is lobed. It is further distinguished from *P. dilimanensis* by the short or absent esophagus and the oral and ventral suckers almost equal in size. While in *P. dilimanensis,* esophagus is always present and the oral sucker is twice larger than the ventral sucker. *P. vespertilionis* is separable from *P. maculosus* in the relatively longer cirrus sac extending posteriorly beyond the ovary in the latter species while in the former species, the cirrus sac does not extend beyond the ovary but terminates at its anterior border. Further, *P. maculosus* occurs in birds while *P. vespertilionis* occurs in bats. A pictorial comparison on the general morphology of the above species is shown in Figs. 1, 4,5, and 6). This is the first report on the occurrence of *P. vespertilionis* in the Philippines constituting a new locality record for the species. This species, including some other species of the genus, have also been reported to infect humans (Guk *et al.,* 2007; Chai, 2019). Members of the genus with known life cycle have been shown to involve dragon flies of the genera *Sympetrum* and *Calopteryx* as intermediate host (Hong *et al.*, 1999; Chae *et al*., 2012). Mammalian host become infected by feeding on adult dragonflies that carry the larval form of the parasite. This may also be the case for *P. vespertilionis* in the Philippines since the bat host is an insectivorous species and that dragonflies of the suborder Anisoptera and order Odonata to which the above genera belong are widely distributed in the country (Hämäläinen and Müller, 1997).The present specimens of *Paralecithodendrium* conform to the diagnosis of that genus in the key to the genera of the family Lecithodendriidae as provided by Lotz and Font (2008). The present materials of *P. longiforme* and *P. ovimagnosum* agree well with the description of these species by Tubangui (1928) (as *Lecithodendrium luzonicum*) and Kifune & Sawada (1986). This is the third report of the two species since these were first and last reported in 1928 and 1986, respectively in the country. Another species, *P. parvouterus* was also reported in the Philippines (Kifune & Sawada, 1986) but was not recovered in the present study. *P. longiforme* differs from both *P. ovimagnosum* and *P. parvouterus* by the shape, size of the body, and various organs. *P. ovimagnosum* and *P. parvouterus* are separable from each other by the body size, occurrence of esophagus, location of the vitellaria, and shape and size of the testes. Members of the family Lecithodendriidae, to which the genus *Paralecithodendrium* belongs, with a known life cycle that involves two-stage intermediate hosts: 1. freshwater snails where cercaria develops and 2. aquatic insects (caddisflies) where metacercaria, the infective form, develops in the insect larva and carried on to its adult stage. Bats become infected by feeding on infected adult caddisfly (Lord *et al.*, 2012; Kudlai *et al*., 2015). Caddisflies belong to the insect order Trichoptera and occur in abundance in the Philippines (Mey, 1998). This work has shown the occurrence, for the first time in the Philippines, of *Plagiorchis vespertilionis*, a trematode species that has been recorded in other countries in bats and in humans, and likewise the presence still of two species of *Paralecithodendrium* which were last recorded in the country three decades ago. All species were fully described and illustrated based on the present materials.

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