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Prevalence, intensity, mean abundance and description of *Phyllodistomum punctati* n. sp. (Digenea: Gorgoderidae) from the urinary bladder of *Channa punctata* (Bloch) from the Western Ghats, India

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Abstract
Phyllodistomum punctati n. sp. infecting urinary bladder of the freshwater fish *Channa punctata* (Bloch) collected from water bodies in the Wayanad region of the Western Ghats during April 2017 to April 2018 is described and illustrated. *Phyllodistomum punctati* n. sp. is new to the genus and is separated from its congeners on the basis of differences in morphology and morphometry. It is named after the host, *C. punctata* (Bloch). The present paper also describes the prevalence (7%), intensity (3.71) and mean abundance (0.26) of infection.

Keywords: *Phyllodistomum punctati* n. sp., fish, *Channa punctata*, Wayanad, Western Ghats

1. Introduction
The Western Ghats is a mega biodiversity region and is rich in freshwater fish diversity and endemism. Dahanukar *et al*. [1] reported that among the 290 species of freshwater fishes belonging to 11 orders, 33 families and 106 genera documented from the Western Ghats region, 189 (65%) are endemic. Freshwater fishes are hosts to taxonomically diverse helminth parasites and infections can significantly affect fish behavior, metabolism, body condition, fecundity and survival [2-4].
Zeder [5] was the first to describe a trematode, *Distomum cygnoides*, from the urinary bladder of frogs and later Braun [6] erected the genus *Phyllodistomum* for *Distomum folium* Olfers, 1816 from the urinary bladder of fishes. *Phyllodistomum*, probably the most diverse genus within the Digenea, has a worldwide distribution with around 120 species [7-10].
The paper describes the morphological features and taxonomic status of a new species of digenetic trematode, *Phyllodistomum punctati* n. sp. infecting urinary bladder of the freshwater fish *Channa punctata* (Bloch) collected from the Wayanad region of Western Ghats. It also describes the intensity, prevalence and mean abundance of infection.

2. Materials and methods
2.1 Study area: The study was carried out in the Wayanad region of the Western Ghats. Western Ghats along with its geographical extension in the wet zone of Sri Lanka are now considered one of the "hottest hotspots" of biodiversity. Wayanad district, part of the Western Ghats, stands on the north-east of Kerala. The map of the study area (Fig. 1) was prepared by using QGIS 2.16.1 software.

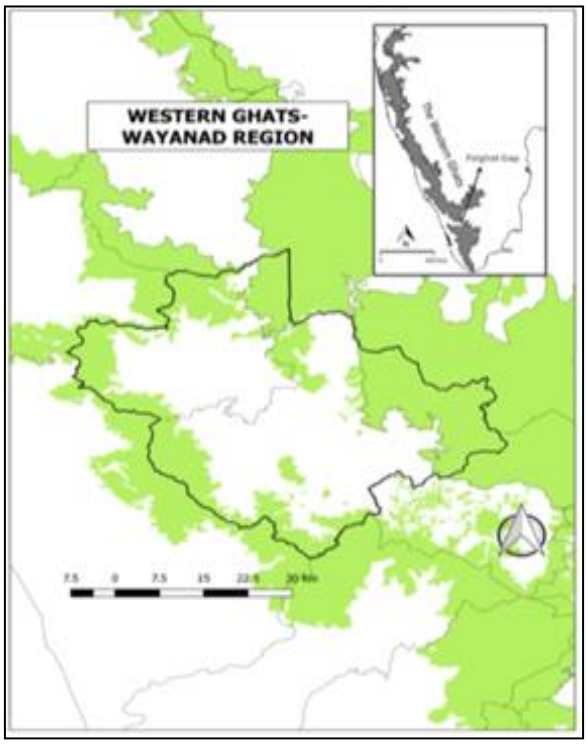

Fig 1: Study area – Western Ghats – Wayanad region.

2.2 Methods: Host specimens were collected from small streams, using sweep net. The collected *C. punctata* were brought alive to the laboratory in suitable containers and maintained in clean glass tanks or aquariums. *C. punctata* were fed occasionally with cooked rice, fish meal or biscuit crumbs. *C. punctata* were sacrificed by cervical rupture and their scales, skin, gills, gill chambers and eyes were examined under Labomed (Luxeo 4Z) stereozoom microscope for both larval and adult digeneans. Skin was removed, and the muscle tissues were macerated to detect the metacercariae and adults, if any. Internal organs like heart, liver, gall bladder, pancreas, intestine, kidney, urinary bladder, swim bladder, gonads and brain were dissected out, placed in separate petri dishes containing 0.75% saline, macerated and examined under the stereozoom microscope. Adults, when present, were carefully transferred to 0.75% saline in a petri dish. The adults were studied under the Nikon ECLIPSE Ni-U phase contrast research microscope (Nikon, Japan) without vital staining or with neutral red or methylene blue stains. Permanent whole mounts of adults were prepared by fixing them in 5% formalin under slight cover glass pressure or by placing the parasite between two slides. Specimens were stained with acetocarmine, following the procedure outlined by Cantwell [11]. Photographs were taken with Nikon Y-TV55 camera and Nikon NIS Elements imaging software. Figures were drawn with Nikon Y-IDT drawing tube and measurements (in µm) were taken with Nikon NIS Elements imaging software.

Prevalence, intensity and mean abundance of infection were measured following Bush *et al*. [12]. Prevalence is the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. It is commonly expressed as percentage. Intensity of infection is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. Abundance is the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected.

3. Results

3.1 *Phyllodistomum punctati* n. sp. (Fig. 2)

Description is based on the holotype and twenty five paratypes. Body spatulate, aspinose, divided into fore-body and hind-body with smooth lateral margins 1513.58-6135.04 x 1032.41-4322.91 (2926.00 x 1979.29). Length to width ratio of body was 1.48:1. Fore-body was conical with a blunt anterior end, 622.33-2576.94 x 631.24-2163.53 (1183.21 x 1135.15). Hind- body was broad, ovoid, 891.25-4002.09 x 1316.80-4322.91 (1742.79 x 1979.24). Oral sucker was terminal, round to oval, 332.54-825.33 x 356.92-767.92 (475.45 x 462.57) in size, larger than ventral sucker; mouth ventral. Ventral sucker was pre-equatorial, almost round, 282.34-733.09 x 294.39-731.09 (441.78 x 456.21). Muscular pharynx was absent. Mouth leads to tubular esophagus, 61.21-374.21 x 52.18-83.90 (151.69 x 56.09) in size. Caeca 927.87-4411.36 x 61.40-223.69 (2173.46 x 133.71) with crinkled margins was found to terminate near the posterior extremity. Excretory vesicle 526.79 – 1564.50 (973.92) was tubular, I-shaped and extending up to ventral sucker. Excretory pore was terminal.

Testes were two in number, intercaecal, lobed and post equatorial. Anterior testis was located posterior to ventral sucker, slightly at the level of ovary but not in close proximity, 129.13-514.40 x 73.06 – 328.00 (295.06 x 187.75) in size. Posterior testis was slightly anterior to caecal end or posterior to ovary, 95.31 – 681.29 x 115.13 – 335.57 (282.63 x 172.37) in size and slightly smaller than anterior testis. Cirrus sac was absent. Seminal vesicle was pre-equatorial, posterior to intestinal bifurcation, slightly anterior to ventral sucker and single chambered. Seminal ducts arising from two testes unite at slightly posterior to seminal vesicle into a common seminal duct and opens to seminal vesicle. In live specimens gonopore could be seen as a small depression at the region of caecal bifurcation, at the anterior part of seminal vesicle (Fig. 3).

Ovary was posterior to ventral sucker, pre testicular, lobed and 113.54 – 434.45 x 103.30 – 377.79 (262.32 x 238.00) in size. Eggs were oval having 20.42-29.96 x 12.79-20.28 (25.06 x 16.39) size. Laurer´s canal was not observed. Vitelline glands were two, lobed and posterior to ventral sucker at the level of ovary. Right vitelline gland was close to ovary and 94.95 – 296.17 x 65.64 – 273.59 (178.84 x 165.44) in size. Left vitelline gland was 98.99 – 326.71 x 53.90 - 308.25 (200.85 x 172.98) in size. Uterus was extensive, covering most of the hind body, filling extra intra-caecal area and intercaecal at fore-body. Metraterm was found to open at gonopore.

3.2 Taxonomic summary

Type specimen: Holotype (No. Z-P/H-F 168) was deposited in the Helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type hosts: *Channa punctata* (Bloch) (Z-FF-2). Deposited in the Ichthyology collections, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type localities: Ozhakkodi Wayanad, Kerala, India.

Site of infection: Urinary bladder.

Period of collection: April 2017 to April 2018.

Prevalence: Seven of 100 *C. punctata* screened were infected and the prevalence of infection was 7%.

Intensity: Twenty six *P. punctati* n. sp. were recovered from seven infected *C. punctata* and hence the intensity was 3.71.
Mean abundance: Twenty six *P. punctati* n. sp. were recovered from 100 *C. punctata* examined and, therefore, the mean abundance was 0.26.
Of the 100 *C. punctata* examined, seven were infected with a total of 26 parasites.

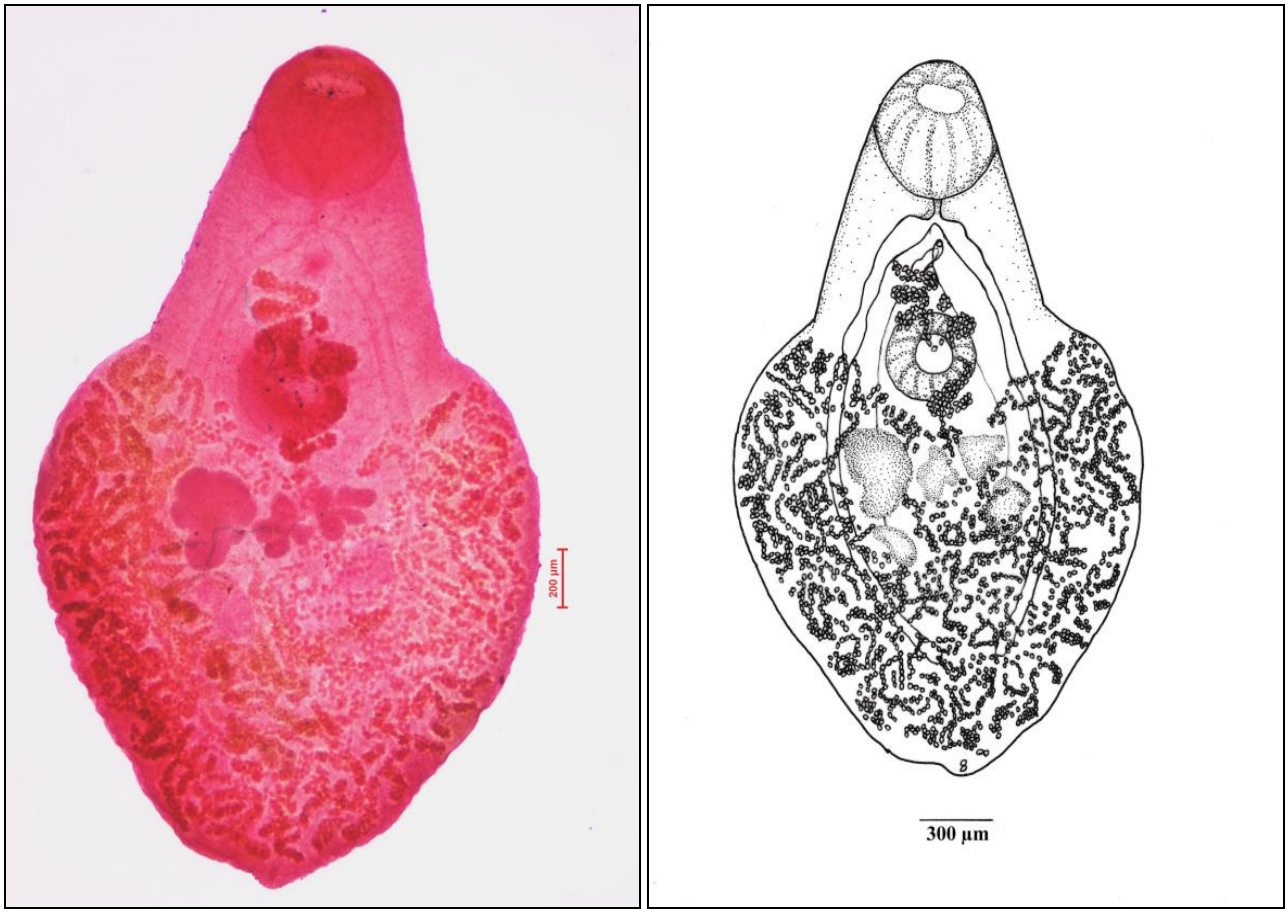

Fig 2: *Phyllodistomum punctati* n. sp., adult (photograph and line drawing).

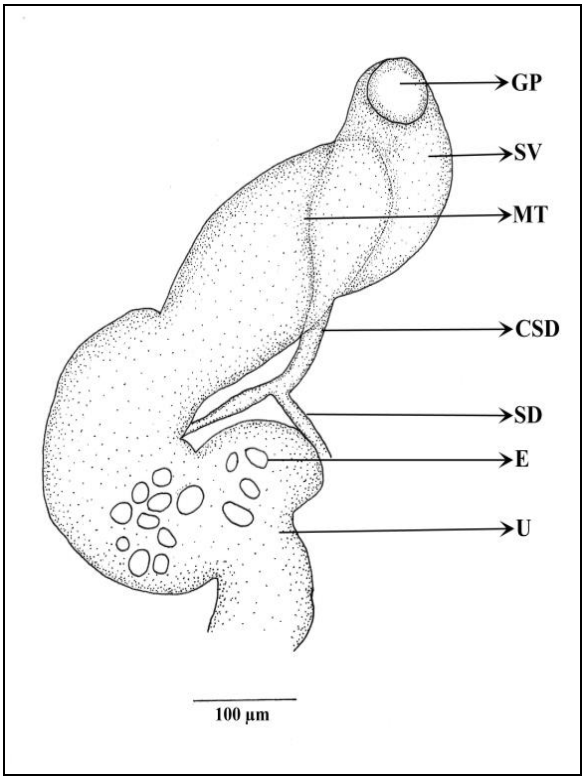

Fig 3: Terminal genitalia of *Phyllodistomum punctati* n. sp. GP – Gonopore; SV – Seminal vesicle; MT – Metraterm; CSD – Common seminal duct; SD – Seminal duct; E – Egg; U – uterus

Table 1: Comparison of morphologic and morphometric characters of *P. tripathi*, *P. cameroni*, *P. betwaensis* and *P. punctati* n. sp.

Characters	*P. tripathi* Motwani and Srivastava, 1961	*P. cameroni* Agrawal, 1966	*P. betwaensis* Sen, 2014	*P. punctati* n. sp.
Body	Dorsoventrally flattened, spatulate, 1550 – 4580 x 740 - 2020 (3065 x 1380	Spatulate, wide just behind testicular region of body (4230 x 2230).	Spatulate, divisible into a narrow tubular, curved fore-body and expanded hind end of the body, 1400 - 1600 x 410 - 430 (1500 x 420) with wavy margin.	Spatulate, aspinose, divided in to fore-body and hind-body with smooth lateral margins 1513.58-6135.04 x 1032.41-4322.91 (2926.00 x 1979.29).
Length to width ratio of body	2.22:1	1.89:1	3.57:1	1.48:1
Fore-body	Cylindrical, narrow, 900 - 2700 x 530 – 800 (1800 x 665)	Narrow and elongate (1800 x 1100)	Narrow, elongated, curved 810 - 830 x 190 – 210 (820 x 200)	Conical with a blunt anterior end, 622.33-2576.94 x 631.24-2163.53 (1183.21 x 1135.15)
Hind-body	Broad and foliate, 690 - 2000 x 740 – 2020 (1345 x 1380). Three pairs of feebly developed semicircular puckerings present on lateral sides	Expanded and nearly circular, (2520 x 2230)	Spatulated, 610 – 630 x 410 - 430 (620 x 420)	Broad, ovoid, with smooth margin 891.25-4002.09 x 1316.80-4322.91 (1742.79 x 1979.24)
Oral Sucker	Terminal, oval, 190 -500 x 190 – 400 (345 x 295)	Globular, terminal, 500 in diameter	Terminal, slightly oval, mouth opening ventrally, 150 - 170 x 110 – 130 (160 x 120)	Terminal, round to oval, mouth ventral 332.54-825.33 x 356.92-767.92 (475.45 x 462.57)
Ventral Sucker	Spherical, larger than oral sucker, 300 - 600 in diameter in anterior third of body	Equal to oral sucker, 500 in diameter located nearly l/3rd of body length from anterior extremity	Oval, larger than oral sucker 230 - 250 x 170 – 190 (240 x 180)	Pre-equatorial, round, smaller than oral sucker 282.34-733.09 x 294.39-731.09 (441.78 x 456.21)
Esophagus	Long, narrow, straight or curved, 110 - 450 in length	Tubular, 190 x 80 in size	Slightly curved, tubular, 90 - 110 x 30 -50 (100 x 40)	Tubular, 61.21-374.21 x 52.18-83.90 (151.69 x 56.09)
Caeca	Broad with crinkled margins and in some immature specimens very close together or apart from each other. Terminate at hind end of body 170 - 500	--	Extends up to the hind end of body	With crinkled margins terminate near posterior extremity 927.87-4411.36 x 61.40-223.69 (2173.46 x 133.71)
Ovary	Submedian, pretesticular and consists of 4-5 lobes, situated just behind left vitelline gland, 100 – 250 x 90 – 250 (175 x 170)	Lobed, pretesticular, 420 x 300 in size and situated on left side of vitelline gland	Oval, post-equatorial, inter-caecal, just behind of right vitelline lobe and parallel to anterior testis, 90 - 110 x 60 – 80 (100 x 70)	Posterior to ventral sucker, pre testicular, intercaecal, lobed 113.54 – 434.45 x 103.30 – 377.79 (262.32 x 238.00)
Anterior Testis	Right testis at level of ovary but not in close proximity, 200 – 480 x 200 – 600 (340 x 400)	Right testis larger than left and 530 x 400 in size	Anterior testis 130 –150 x 100 – 120 (140 x 110) larger than posterior testis and parallel to ovary.	Anterior testis posterior to ventral sucker, slightly at level of ovary but not in close proximity 129.13-514.40 x 73.06 – 328.00 (295.06 x 187.75).
Posterior Testis	Left testis slightly larger than right located near termination of ceca or slightly anterior to it, 250 – 550 x 200 – 500 (400 x 350)	Left testis 440 x 420 in size	Posterior testis 110 – 130 x 80 - 100 (120 x 90)	Posterior testis slightly anterior to termination of caeca or posterior to ovary 95.31 – 681.29 x 115.13 – 335.57 (282.63 x 172.37), slightly smaller than anterior testes
Eggs	Oval, non operculated, 51.20 - 75.20 x 25.90 – 49.20 (63.20 x 37.55)	Oval, non operculated, 39.1 – 52.2 x 19.4 – 32.5 (45.65 x 51.90) in size	Oval, non-operculated, 20 – 40 x 10 – 20 (30 x 15)	Oval 20.42-29.96 x 12.79-20.28 (25.06 x 16.39)
Vitelline Gland	Two, bilobed masses lying asymmetrically on both sides of body just behind ventral sucker close infront of ovary ; right vitelline gland 30 - 120 X 110 – 310 (75 x 210) and left vitelline gland 30 -100 x 110 - 320 (65 x 215) in size	Consist of two divided follicles lying behind ventral sucker one on either side of ootype; right vitelline gland 100 x 28 and left 100 x 29 in size	Two, posterior-lateral to ventral sucker, oval, and rarely lobed. Right vitelline lobe 10 – 80 x 30 - 50 (45 x 40) is larger than left vitelline lobe 50 – 70 x 20 – 40 (60 x 30)	Two, lobed, posterior to ventral sucker, at the level of ovary. Right vitelline gland close to ovary, 94.95 – 296.17 x 65.64 – 273.59 (178.84 x 165.44) in size and left vitelline gland 98.99 – 326.71 x 53.90 - 308.25 (200.85 x 172.98)

4. Discussion

The genus *Phyllodistomum* has been reported from numerous species of freshwater & marine fishes and amphibians throughout the world and it comprises of more than 120 species [9, 13]. Sixteen species of the genus *Phyllodistomum* have been described so far from freshwater fishes of India;

these are *P. spatulaeforme* Odhner, 1902; *P. lewisi* Srivastava, 1938; *P. macronium* (Dayal, 1938) Yamaguti, 1958; *P. callichrius* (Dayal, 1942); *P. vachius* Dayal, 1949; *P. loossi* Kaw, 1950; *P. singhiai* Gupta, 1951; *P. vittatusi* Gupta, 1955; *P. parorchium* Jaiswal, 1957; *P. indianum* Jaiswal, 1957; *P. chauhani* Motwani and Srivastava, 1961; *P. tripathi* Motwani and Srivastava, 1961; *P. srivastavi* Rai, 1964; *P. cameroni* Agarwal, 1966; *P. triangulate* Sarwat, 2011 and *P. betwaensis* Sen, 2014.

The *P. punctati* n. sp. exhibits similarities with *P. tripathi, P. cameroni* and *P. betwaensis*. It differs from *P. tripathi* in various morphological features and morphometry like length to width ratio of body, size of oral sucker, size and shape of ventral sucker, length of caeca, position and size of ovary, size of testes and vitelline gland [14].

The present species deserves comparison with *P. cameroni* also (Table 1). In spite of the similarities, it differs from *P. cameroni* in many morphological features and morphometry including the ratio of body length to width, size and shape of oral sucker, size of ventral sucker, length of esophagus, size of testes, eggs and vitelline gland [15].

The present species shows some similarities with *P. betwaensis* too. It differs from *P. betwaensis* in many morphological features and morphometry like size of body and oral sucker, size and shape of ventral sucker, length of esophagus, size and position of ovary, size of testes and vitelline gland [16].

5. Conclusion

Fishes serve as hosts to a wide range of taxonomically diverse parasites including Protozoans, Helminthes and Arthropodes. Among the helminth parasites digenetic trematodes are the most important ones. Trematode parasites have complex life cycles, requiring multiple hosts. The life cycle of these digenetic trematodes generally follows the exploitation of different host organisms, including both vertebrates and invertebrates. Through their life cycles these parasites are functionally coupled with the surrounding free-living diversity of vertebrate and invertebrate animals. Fishes serve as both intermediate and definitive hosts for trematodes. If these parasites are present in fishes, then one can infer that other hosts of these parasites must also be present in that ecosystem. Thus the presence of parasites in fishes may be useful indicators of species diversity in that region. After elucidating and establishing the life cycles of trematode parasites of fishes, the larval trematodes can be universally taken as indicators of fish diversity. The present investigation is a stepping stone to achieve that goal. This study is a part of a project on the studies on trematode parasites infecting Western Ghats, Wayanad region.

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7. References

1. Dahanukar N, Raghavan R, Ali A, Abraham R, Shaji C. The status and distribution of freshwater fishes of the Western Ghats, 21–48. In: (Compilers. Molur S, Smith KG, Daniel BA, Darwall WRT) International Union for Conservation of Nature (IUCN) Gland, Switzerland and Zoo Outreach Organization (ZOO), Coimbatore, 2011, 21-48.
2. Barber I, Hoare D, Krause J. Effects of parasites on fish behaviour: A review and evolutionary perspective. Reviews in Fish Biology and Fisheries. 2000; 10:131-165.
3. Lafferty KD. Ecosystem consequences of fish parasites. Journal of Fish Biology. 2008; 73:2083-2093.
4. Seppanen E, Kuukka H, Voutilainen A, Huuskonen H, Peuhkuri N. Metabolic depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. Journal of Fish Biology. 2009; 74:553-561.
5. Zeder JGH. Erster Nachtrag zur Naturegeschichte der Eingeweidewiirmer, mit Zusatzen und Ammerkungen herausgegeben. Leipzig, xx +, 1800, 320.
6. Braun M. Ein neues Distomum aus Porphyrio. Zoologischer Anzeiger. 1899; 22:1-4.
7. Cribb TH, Chisholm LA, Bray RA. Diversity in the Monogenea and Digenea: does lifestyle matter?. International Journal of Parasitology. 2002; 32:321-328.
8. Helt J, Janovy JJr, Ubelaker J. *Phyllodistomum funduli* n. sp. (Trematodag: Orgoderidae) from *Fundulus sciadicus* cope from Cedar Creek in Western Nebraska. Journal of Parasitology. 2003; 89(2):346-350.
9. Mendoza-Garfias B, Pérez-Ponce De León G. *Phyllodistomum centropomi* sp. n. (Digenea: Gorgoderidae), a parasite of the fat snook, *Centropomus parallelus* (Osteichthyes: Centropomidae), in the Papaloapan River at Tlacotalpan, Veracruz State, Mexico. Zootaxa. 2005; 1056:43-51.
10. Pérez-Ponce De León G, Aquinoa AM, Mendoza-Garfias B. Two new species of *Phyllodistomum* Braun, 1899 (Digenea: Gorgoderidae), from freshwater fishes (Cyprinodontiformes: Goodeidae: Goodeinae) in central Mexico: An integrative taxonomy approach using morphology, ultrastructure and molecular phylogenetics. Zootaxa. 2015; 1:087-099.
11. Cantwell GE. Methods for invertebrates. In: (Eds. Clark G.) Staining procedures. Baltimore, Williams and Wilkins, 1981, 255-280.
12. Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis *et al*. Revisited. Journal of Parasitology. 1997; 83(4):575-583.
13. Cribb T. Studies on gorgoderid digeneans from Australian and Asian freshwater fishes. Journal of Natural History. 1987; 21:1129-1153.
14. Motwani MP, Srivastava CV. On the phyllodistomes from the urinary bladder of siluroid fishes. (Trematoda: Gorgoderiidae). Indian Journal of Helminthology. 1961; 13:93-99.
15. Agarwal V. Studies on some trematode parasites of freshwater fishes from Lucknow. Annals of Parasitology. 1966; 24:217-231.
16. Sen JM. On a New Species of *Phyllodistomum* Braun, 1899 (Digenea: Gorgoderidae), A Parasite of Freshwater Fish, *Channa Punctatus* (BL.) From Betwa River, Bundelkhand Region Jhansi, U.P, India. Current World Environment. 2014; 9(1):207-209.