Metazoan parasite fauna of *Clarias batrachus* (Linn.) of river Godavari with description of a new species of digenean, *Phyllodistomum batrachii* sp. nov.

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ABSTRACT

A survey has been made on the parasites of *Clarias batrachus* (Linn.) from the river Godavari, Rajahmundry during August 2005 to September 2007. A total of 108 host fishes were examined during the study period of which 70 were infected with numerous metazoan parasites. A total of 10 species of parasites has been recorded of which 5 are digeneans (*Allocreadium handiai*, *Orientocreadium batrachoides*, *Emoleptalea proteopora*, *Astiotrema reniferum* and a new species, *Phyllodistomum batrachii*; 3 cestodes, *Lytocestus indicus*, *L. longicollis* and *L. birmanicus*; an acanthocephalan- juvenile of *Centrorhynchus batrachus* and a copepod, *Lamproglena chinensis*. *Phyllodistomum batrachii* sp. nov. is described from the urinary bladders of the host and described in detail. It differs from the already described species in body size and shape, sucker ratio, shape of ovary and vitellaria and distribution of uterine coils in the entire hind body. *Clarias batrachus* is the new host and the geographical record for the juvenile of an Acanthocephalan, *Centrorhynchus batrachus* and copepod, *Lamproglena chinensis*.

Keywords: *Clarias batrachus*, *Phyllodistomum batrachii*, *Lytocestus longicollis*, *L. birmanicus*

INTRODUCTION

Fish serve as a potential source of animal protein for man [1-3]. About 40% of the protein diet of 2/3rd of the entire global population comes from fish [4,5]. Majority of the fishes serve as an intermediate hosts for many parasites, which reduces the food value of the fish and cause mass mortality [6]. Clariid fishes constitute an important group of fishes in India due to their abundant occurrence, cosmopolitan distribution and high commercial value. These fishes are locally known as 'marpu' and are highly relished in southern India due to their rich protein content and delicacy. *Clarias batrachus* (Linn.) serves as an imperative host as they carry a range of host-specific parasites. Vast amount literature is available on the parasite fauna of clariid fishes [7-28]. In the present survey, only 10 parasitic species from the 4 parasitic groups, digeneans, cestodes, acanthocephalans and copepods were obtained and described. A new species of digenean, *Phyllodistomum batrachii* n. sp. is also described in detail.

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MATERIALS AND METHODS

Research samples were collected during 2004 to 2006 from river Godavari, Rajahmundry. A total of 108 fishes were examined thoroughly for the metazoan parasites. Parasitological examination of the major organs like gills, liver, body cavity, stomach and intestine are was under a dissecting microscope. Body tissues of the host were alienated, washed in physiological saline to remove excess mucus and were cut open with a longitudinal incision. Parasites were carefully collected and placed in cavity blocks filled with saline solution. Copepod parasites collected from gills were fixed in 10% formalin and then placed in cavity blocks with a few drops of lactic acid for 12–24 hours for clearing and identification. Trematodes, cestodes and acanthocephalans were flattened between two slides or under slight pressure of slide and coverslip, post-fixed in A.F.A (Alcohol-85ml, Formalin-10ml and acetic acid-5ml) and stained with alum carmine [29,30]. Conventional techniques were employed for permanent whole mount preparations. Figures were drawn with the aid of camera lucida. Measurements are given in millimeters. Holotypes and paratypes of the parasites are placed in the Museum of Zoology Department, Andhra University, Visakhapatnam (ZDAU).

RESULTS AND DISCUSSION

During the investigation period, a total of 10 parasitic species from four groups, i.e., digeneans-*Allocreadium handiai* Pande, 1937; *Orientocreadium batrachoides* Tubangui, 1930; *Emoleptalea proteopora* Thomas, 1958 and *Astiotrema reniferum* (Looss, 1898) Stossich, 1904 were identified. A new species, *Phyllodistomum batrachii* sp. nov. is described and illustrated. Only 3 species of cestodes of the family Lytocestidae, *L. indicus*, *L. longicollis* and *L. birmanicus*. *Clarias batrachus* is a new host and geographical record for the juvenile of an acanthocephalan, *Centrorhynchus batrachus* and a copepod, *Lamproglena chinensis* and their infection prevalence is represented in table 1.

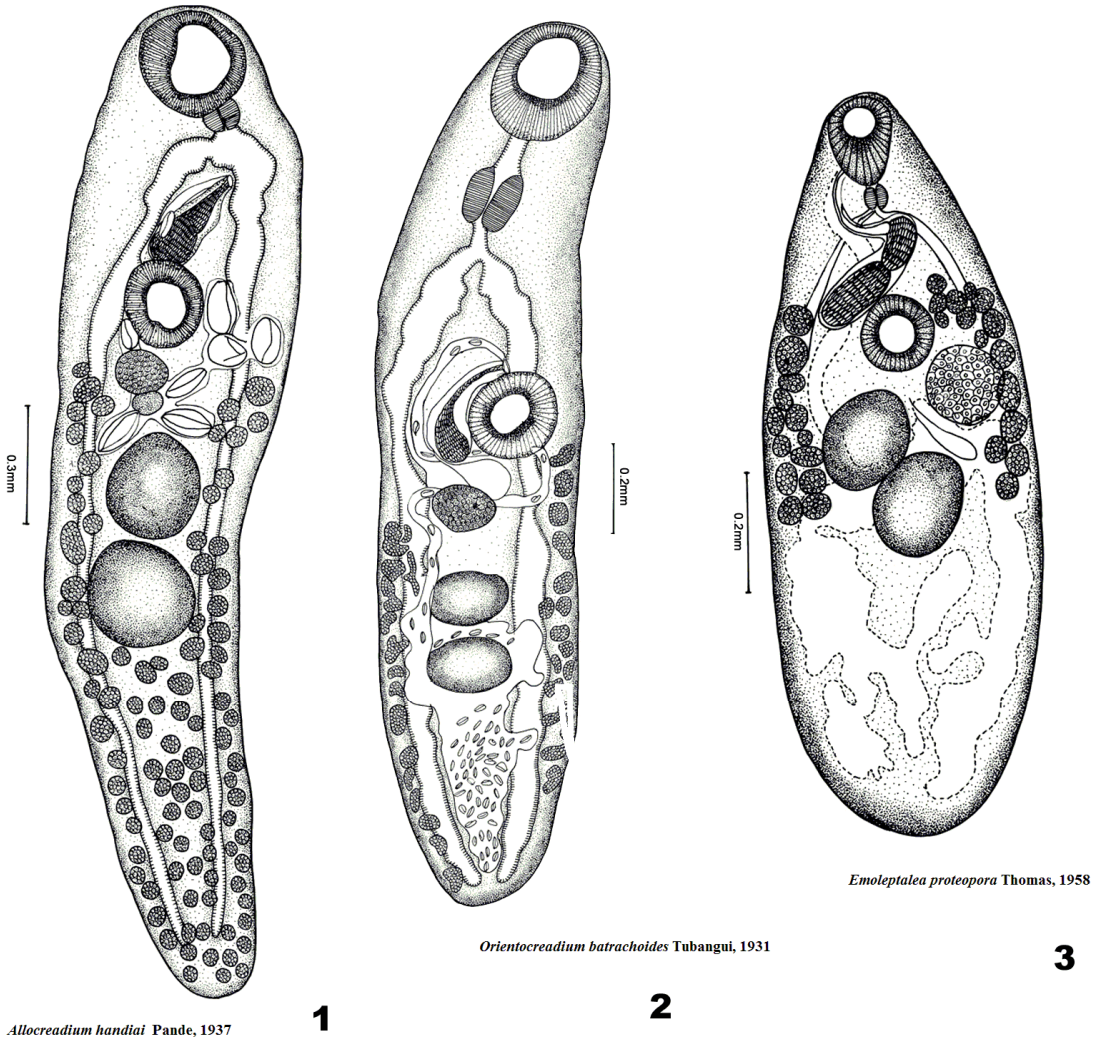
Table 1. Parasites of *Clarias batrachus* from river Godavari, Rajahmundry.

Parasite	Number of fish examined	Number of infected fish	Number of parasites	Site of infection	Prevalence (%)	Mean Intensity	Mean abundance
Allocreadium handiai	108	12	25	Intestine	11.10	2.08	0.230
Orientocreadium batrachoides	108	48	79	Intestine	44.40	0.44	0.730
Emoleptalea proteopora	108	2	2	Intestine	1.85	1.00	0.018
Astiotrema reniferum	108	1	1	Intestine	0.92	0.00	0.009
Phyllodistomum batrachii sp. nov.	108	3	4	Urinary bladder	2.80	1.33	0.037
Lytocestus indicus	108	15	25	Intestine	13.80	1.66	0.230
L. birmanicus	108	2	4	Intestine	1.85	2.00	0.037
L. longicollis	108	2	3	Intestine	1.85	1.50	0.027
Centrorhynchus batrachus Juvenile	108	1	1	Intestine	0.92	1.00	0.009
Lamproglena chinensis	108	4	5	Gills	3.70	1.25	0.005

Allocreadium handiai Pande, 1937 (Figure 1)

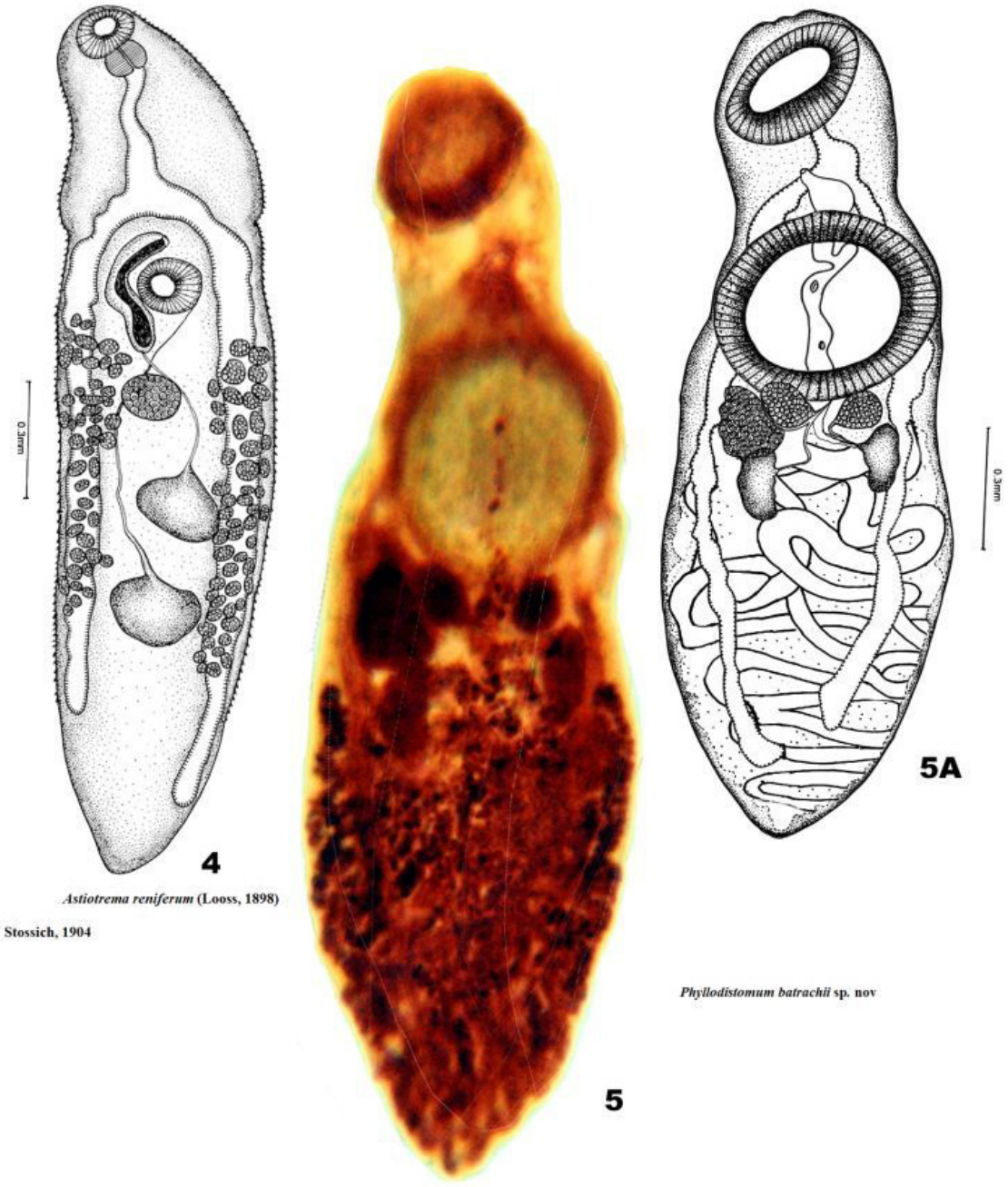
Allocreadium handiai was found in the intestine and have 11.1% infestation prevalence. Body measures 2.21-2.70 × 0.38-0.66, oral sucker 0.17-0.25 × 0.17-0.25; pharynx 0.07-0.09 × 0.06-0.10; oesophagus 0.02-0.03; acetabulum 0.17-0.22 × 0.13-0.22; sucker ratio 1:1.2. Anterior testis 0.17-

0.25 × 0.19-0.26; posterior testis 0.26-0.30 × 0.17-0.25; cirrus sac 0.20-0.23 × 0.10-0.15; ovary 0.15-0.17 × 0.10-0.18; vitelline follicles 0.08-0.10 × 0.06-0.09. Eggs 0.08-0.10 × 0.04-0.06.

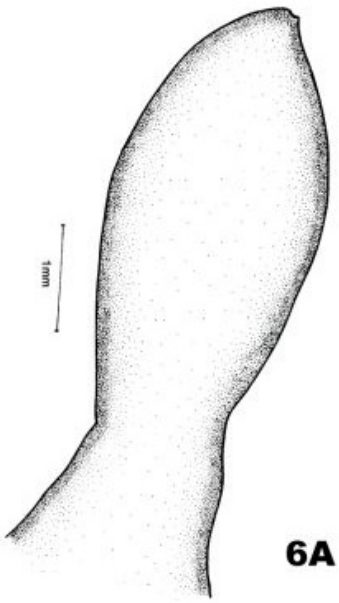


Orientocreadium batrachoides Tubangui, 1931 (Figure 2)
Orientocreadium batrachoides was found fairly in the intestines of 48 out of 108 fishes examined. Body 2.21-2.4 × 0.5-0.6; oral sucker 0.20-0.22 × 0.22-0.24; prepharynx 0.03-0.04; pharynx 0.05-0.07 × 0.07-0.08; oesophagus 0.01-0.02; acetabulum 0.15-0.19 × 0.15-0.19. testes; 0.21-0.24 × 0.22-0.24; cirrus sac 0.13-0.17 × 0.10-0.17; ovary 0.15 × 0.13-0.16; eggs 0.06-0.08 × 0.06.

Emoleptalea proteopora Thomas, 1958 (Figure 3)
Only two fishes were infected with the parasites. Body 0.65-0.72 × 0.26; oral sucker 0.07-0.10 × 0.07-0.08; pharynx 0.02 × 0.02-0.03; oesophagus Acetabulum 0.07-0.09 × 0.06-0.08; testes 0.07-0.08 × 0.08-0.09; Cirrus sac 0.11-0.19 × 0.03-0.04; ovary 0.06-0.07 × 0.07-0.08 and eggs 0.01-0.02 × 0.01.



Astiotrema reniferum (Looss, 1898) Stossich, 1904 (Figure 4)
Only one parasite was obtained from the intestine of *C. batrachus* out of 108 fish examined. Body 2.31 × 0.49, oral sucker 0.13 × 0.13, prepharynx 0.03 × 0.08 long, pharynx 0.08 × 0.10, oesophagus 0.32 × 0.05, acetabulum 0.13 × 0.16, anterior testis 0.17 × 0.18, posterior 0.16 × 0.21. Cirrus sac 0.33 and ovary 0.11 × 0.13.



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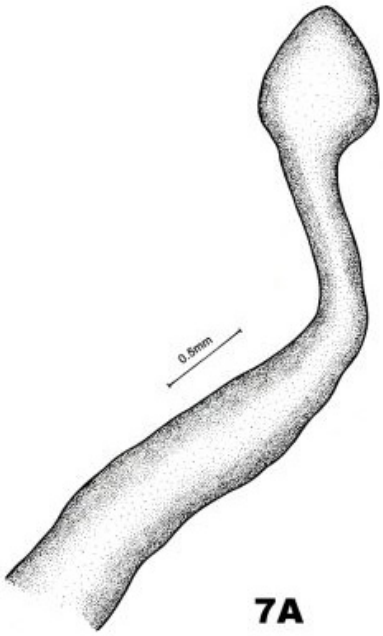

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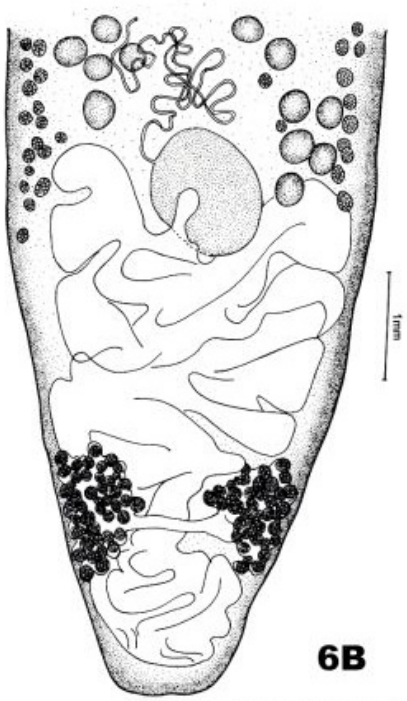

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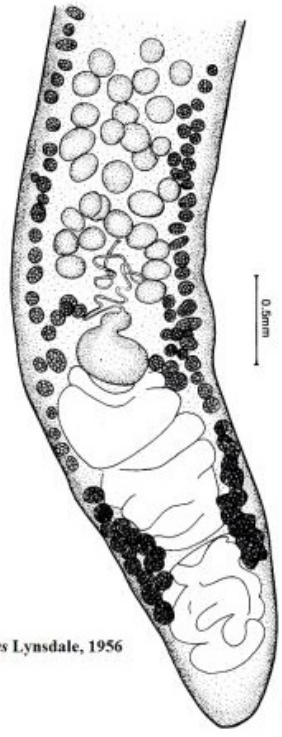
Lytocestus indicus (Moghe, 1925) Yamaguti, 1959



Lytocestus birmanicus Lynsdale, 1956

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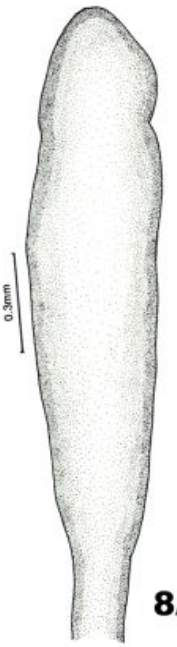

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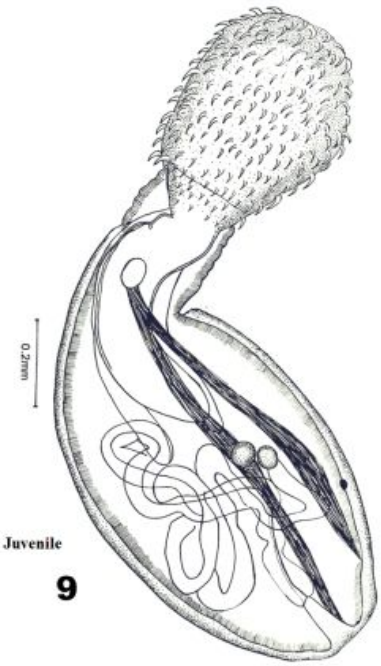

Centrorhynchus batrachus Das, 1952- Juvenile

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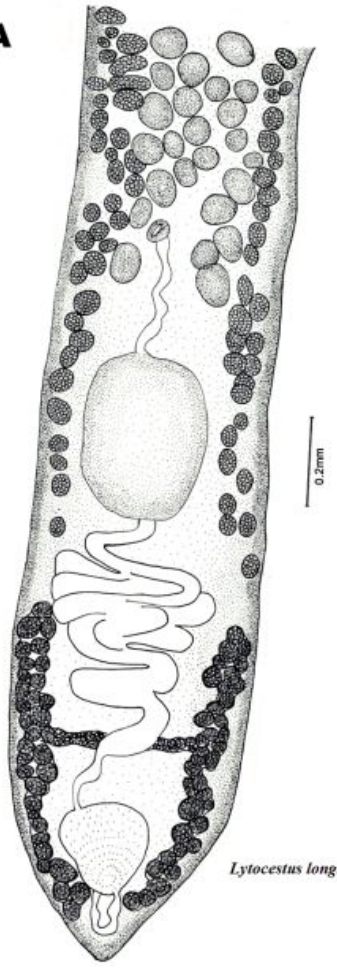

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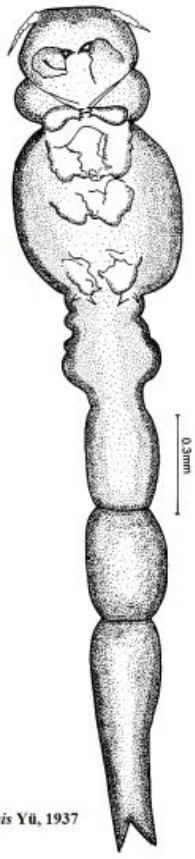
Lytocestus longicollis Ramadevi, 1973



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Lamproglena chinensis Yü, 1937

Phyllodistomum batrachii sp. nov (Figure 5, 5a)
Family : Gorgoderidae (Looss, 1899) Looss, 1901
Sub family : Phyllodistominae (Nybelin, 1926) Yamaguti, 1958
Genus : Phyllodistomum Braun, 1899
: *Phyllodistomum batrachii* n. sp.
Only 4 specimens were obtained from the urinary bladders of 3 infected fish. Body small, creamy-white in colour with slightly narrower anterior end and a broader posterior end. Body measures 1.32-1.35 in length, 0.22-0.26 in width at the narrow anterior region and 0.47-0.51 in width at the broader posterior extremity. Oral sucker sub- terminal, smaller than acetabulum and measures 0.17-0.19 × 0.21-0.25. Pharynx absent. Oesophagus short, measures 0.03-0.05 × 0.04-0.05. Intestinal bifurcation pre-acetabular, intestinal caeca sinous, terminating near the posterior e×tremity and not e×tending past uterus. Acetabulum large, round and measures 0.31-0.36 × 0.30-0.38. Sucker ratio is 1: 1.94 and distance between the two suckers is 0.140. Testes two, intercaecal, post-ovarian, post-acetabular, slightly lobed, located in the broader part of the hind body and measure 0.12-0.14 × 0.06-0.07. Seminal vesicle bipartite, pre-acetabular, globular. Cirrus sac absent. Ovary oval, large, post-acetabular and measures 0.13 × 0.12. Vitelline glands paired, post-acetabular and oval. Right vitelline gland measures 0.09 × 0.08 while the left 0.07 × 0.07. Uterus extensive, occupying most of the hind-body and extends forward to form metraterm which finally opens into genital atrium. Genital atrium median, opens lateral to oesophagus. Eggs small, round, light brown in colour and measure 0.015 × 0.01.
Deposition of specimens: Holotype and paratypes deposited in Zoology Department, Andhra University, Visakhapatnam (ZDAU).
Etymology: Named after the species name of the host.

The genus *Phyllodistomum* was erected by Braun with *P. folium* Olfers, 1816 as type species [31]. Yamaguti described two genera: *Phyllodistomum* Braun, 1899 and *Gorgotrema* Dayal, 1938 on the basis of number of testicular follicles [32]. Dawes included the species from freshwater fishes under *Phyllodistomum* and those, from amphibia under Gorgoderidae Looss, 1899 [33]. The species of *Phyllodistomum* are typically found in the urinary bladder of a variety of ectothermic vertebrates, primarily fishes and amphibians. There are many species reported from various freshwater fishes. From India, it was first reported by Bhalerao from the intestine of *Belone cancila* and *Ophiocephalus marulius* and the stomach of *Mastacembelus armatus* [34]. He appended a key for the ready identification of the then known 27 valid species. Later, there are many reports from India from various freshwater fishes like *Belone cancila*, *Glossogobius giurus*, *Mastacembelus armatus*, *Mystus tengra*, *M. seenghali* and *Rita rita* [21,35-43]. Prudhoe & Hussey reported *P. vanderwaali* and Bookmer reported *P. bavuri* from *Clarias gariepinus* from South Africa [44,45]. However, present parasites differ in many characters from these two species. Present parasites are compared with some of the related species like *P. parorchium* Jaiswal, 1957; *P. tripathii* Motwani and Srivastava, 1961; *P. vanderwaali* Prudhoe & Hussey, 1977 and *P. bavuri* Bookmer, 1984. Present parasites resemble *P. parorchium* in the sucker ratio, globular seminal vesicle and body size but differ in many characters like body shape, shape and position of testes, ovary uterus and shape of vitellaria. Hind body is broad and leaf-like while it is slightly broad and entire in the present parasites. Vitellaria is bilobed in *P. parorchium* while they are round in the present parasites. Uterus is strictly intercaecal in *P. parorchium* while it occupies the entire hind body in the present parasites. *P. tripathii* resemble with present parasites in having a large ventral sucker than oral sucker but differ in many characters like body shape, sucker ratio, arrangement of testes and shape of seminal vesicle. Present parasites come closer to *P. bavuri* in the position and shape of vitellaria and also ovary but differ in sucker ratio, position and shape of testes, egg size and position of genital atrium. Testes irregularly lobed and are larger than ovary in *P. bavuri*, however, testes are

lobed with slight indentations and smaller than ovary in the present species. Eggs of *P. bavuri* slightly larger than eggs of the present parasites. Present parasites resemble *P. vanderwaali* in the position of testes however, differ from it in many characters like sucker ratio, oesophagus, position of genital atrium, shape of ovary and testes, egg size and position of uterus. Uterus is strictly intercaecal in position in *P. vanderwaali* but occupies the entire hind body except the gonads in the present parasites. A table comparing the present species with the above four species is represented in table 2. Basing on the differentiating characters they are considered as new species and named as *Phyllodistomum batrachii* n. sp.

Table 2. Comparison of *Phyllodistomum* nov. sp. with other valid species of the genus.

Characters	*P. parorchium* Jaiswal, 1957	*P. tripathii* Motwani & Srivastava, 1961	*P.vanderwaali* Prudhoe & Hussey, 1977	*P. bavuri* Bookmer, 1984	Present species
Host	*Glossogobius giurus*	*M. armatus, E. vacha*	*Clarias gariepinus*	*Clarias gariepinus*	*Clarias batrachus*
Body shape	Slightly narrow anteriorly and leaf-like posteriorly	Narrow anteriorly and broad smooth ends posteriorly	Pear-shaped	Slightly narrow and leaf-like at posterior ends	Slightly narrow anteriorly and bluntly fusiform at posterior end of body
Body size	1.00-1.25 mm	0.93-1.57mm	1.9-2.5 mm	3.49-5.18 mm	1.32-1.35 mm
Oral sucker	0.10-0.12 × 0.12-0.13	0.10-0.16 × 0.10-0.15	0.23-0.25 × 0.23-0.25	0.27-0.36 × 0.26-0.36	0.17-0.19 × 0.21-0.25
Ventral sucker	0.18-0.19 × 0.18-0.19	0.15-0.23 × 0.14-0.23	0.35-0.40 × 0.34-0.43	0.41-0.63 × 0.40-0.64	0.31-0.36 × 0.33-0.38
Sucker ratio	1:1.8	1:1.5	1:1.0	1:1.5	1:1.94
Testes	Anterior larger than posterior	Oblique	Slightly tandem, lobed	Large, irregularly lobed, opposed to each other	Equatorial, shape irregular only slight indenatations
Ovary	Single	Single, small	Shallowly indented and irregularly lobed	Round, sometimes weakly trilobed	Larger than testes, entire
Vitellaria	Bilobed, post-acetabular	Bilobed	Bilobed, slightly away from ventral sucker	Compact or slightly lobed	Round, very close to ventral sucker
Seminal vesicle	Globular	Sac-like	-	-	Globular
Uterus	Strictly intercaecal	Intercaecal	Strictly Intercaecal	Intercaecal and only very few coils extend laterally and beyond caeca	Intercaecal and extracaecal, occupies the entire hindody

Lytocestus indicus (Moghe, 1925) Yamaguti, 1959 (Figure 6a,b)
25 parasites are found infecting 15 out of 108 examined *C. batrachus*. Worms 24.7-29.0 × 2.52-2.63; Scolex 0.65-0.75 × 1.20-1.29; testicular follicles 0.14-0.19 × 0.20-0.27; cirrus sac 0.92-1.02 × 0.4-0.60; Vitellarian follicules 0.06-0.10 × 0.12-0.16; ovarian follicles 0.10-0.14 and eggs 0.05-0.06 × 0.02-0.03.

Lytocestus birmanicus Lynsdale, 1956 (Figure 7a,b)
Only two out of 108 examined *C. batrachus* were infected with 4 parasites. Worms 8.15-10.05 × 0.71-0.84; scolex 0.48-0.60 × 0.39-0.60; neck 1.98-3.68 × 0.16-0.36; testes 0.06-0.09 × 0.05-0.07; cirrus sac 0.21-0.31 × 0.16-0.26; ovary 0.05-0.07× 0.04-0.09; vitellarian follicles 0.04-0.06 × 0.04-0.07 and eggs 0.04 × 0.02.

Lytocestus longicollis Ramadevi, 1973 (Figure 8a,b)
Only 3 specimens were obtained from the intestines of 2 infected fish. Body 10.52-15.5 × 0.60-0.65; Scolex 0.92-0.99 × .034-0.58; neck 5.00-5.42; testes 0.06-0.08 × 0.07-0.09; ovarian follicles 0.03-0.06 × 0.03-0.05; Vitellarian follicles 0.03-0.07 × 0.03-0.04.

Centrorhynchus batrachus Das, 1952-Juvenile (Figure 9)
A rare parasite was obtained from the intestine of the *C. batrachus*. Body 2.63 × 0.33; proboscis 0.27 × 0.26; leminsci 0.40 × 0.03; testes 0.04 × 0.04.

Lamproglena chinensis Yü, 1937 (Figure 10)
Only 5 parasites were found infesting the gills of 4 out of 108 fish examined. Body 2.5-2.9 × 0.25-0.38; Cephalon 0.24-0.28 × 0.35-0.36; neck 0.20-0.22 × 0.36-0.38; trunk 0.62-0.63 × 0.43-0.45; fifth thoracic segment 0.06-0.08 × 0.23-0.26; genital segment 0.22-0.25 × 0.30-0.32; abdomen basal and middle 0.31-0.33 × 0.22-0.24 and distal 0.52-0.55 × 0.19-0.20.

Clarias batrachus is a commonly available species in the river Godavari, Rajahmundy and their metazoan parasite fauna is investigated. *Allocreadium handiai* is the first member of the Indian species and has been reported from *Ophiocephalus punctatus* by Pande [46,47]. Mehra reviewed the classification of Allocreadioidea Nicoll, 1937 and recognized 7 Indian species as valid [48,49]. Madhavi depicted the life-cycle of *A. handiai* and she synonymized *A. nicolli* Pande, 1937 from *Glossogobius giurus* and *A. heteropneustis* Agrawal, 1964 as *A. handiai* [50]. Khan and Bilquees reported *A. kalriai* from *Channa striatus* [51] and in the present study this parasite was reported from *C. batrachus*.

O. batrachoides is a commonly occurring parasite of the *C. batrachus* with 44.4% of infestation prevalence. The genus *Orientocreadium* with *O. batrachoides* as its type species from *Clarias batrachus* was proposed by from Philippinnes [52]. Later, 16 species are listed under this genus by Yamaguti of which 13 are reported from India [53]. Most of them are reported from *Clarias sp*. Beverley-Burton synonymized six species, *O. indicum* Pande, 1934; *O. barabankiae* Dayal, 1938, *O. dayali* Yamaguti, 1958, *O. secundum* Dayal, 1949, *O. mahendrai* Gupta, 1951, *O. philippai* Gupta, 1951 and *O. vermai* Gupta, 1951 with *O. batrachoides* [54].

E. proteroptera is a rare parasite obtained from the intestine of *C. batrachus* with only 1.85% prevalence. The genus *Emoleptalea* was erected by Looss with *E. exilis* as its type-species from *Bagrus bayad* [55]. Later, there were four reports of species from the genus namely, *E. dollfusi* Srivastava, 1960; *E. loossi* Srivastava, 1960 from *Saccobrancus* (*Heteropneustes*) *fossilis*; *E. proteroptera* Thomas, 1958 from *Clarias senegalensis* and *E. synodontidos* Dollfus, 1950 from *Synodontis notatus ocellatus*.

Astiotrema reniferum is a rare parasite obtained in the present study. *Astiotrema reniferum* as type species was proposed by Looss from turtle, *Trionyx* [55]. Yeh and Fotedar reviewed the genus and listed 21 species of which only four are considered to be valid, *A. reniferum* (Looss, 1898) Stossich, 1904, *A. implementum* Looss, 1898, *A.monticelli* Stossich, 1904 and *A. odhneri* Bhalerao,1937 and gave the key for identification of recognized species [56]. Two species *A. lobiorchis* and *A. mehrai* repoted by Tiwari from India was later synonymized with *A. reniferum* by Siddiqi [57,58]. Beverley-Burton reported *A. reniferum* from *Clarias mossambicus* from Rhodesia and Agarwal reported it from *Heteropneustus fossilis* from India [35,54]. Later, El-Naggar et al. redescribed *A. reniferum* from *C. lazera* in Egypt [59].

In the present investigation, *Lytocestus indicus* showed 13.8% infestation prevalence. The genus *Lytocestus* was first proposed by Cohn with *C. adhaerens* as its type-species from the intestine of *Clarias fuscus* from Hong Kong [60]. There are many reports of species of this genus from various parts of the world. Moghe first proposed the species *Caryophyllaeus indicus* from *Clarias batrachus* [61]. But later, basing on Woodland's comments, Moghe corrected his description and renamed *Caryophyllaeus indicus* as *Lytocestus indicus* [62,63]. Chakravarthy and Tandon, and Rama Devi also recorded this species from north-eastern and southern India respectively [64,65]. There are reports of few more species under this genus by Singh and Tandon et al. from India [66,67].

The species *Lytocestus birmanicus* was first proposed by Lynsdale from the intestine of *Clarias batrachus* from Rangoon [68]. Later, Chakravarthy and Tandon reported the same species from India as new geographic record [64]. Present parasites are in conformity with those of Lynsdale except for minute variations in their measurements and in the number of testes [68]. The species *Lytocestus longicollis* was first erected by Rama Devi from the intestine of *Clarias batrachus* from India [65]. Chakravarthy and Tandon reported this species from north-eastern region of India [64]. Present parasites resemble *L. longicollis* in most of the characters except for minute variations in their measurements and number of testes.

Centrorhynchus batrachus was first erected by Das for larval form (juvenile) from the body cavity of *Rana tigrina* (Daud) [69]. Jain reported juveniles of *Centrorhynchus batrachus* from a variety of hosts including fishes, amphibians, reptiles, birds and mammals of which birds are considered as definitive hosts, while the other hosts are considered as intermediate hosts [70]. Chowhan and Khera also recorded these parasites from *Bagarius bagarius*, *Channa punctatus* and *Puntius sarana* [71]. In the present study, the parasite was obtained from *Clarias batrachus*. Hence, it is a new host record for the parasite.

Lamproglena chinensis was proposed by Yü [72]. Yamaguti described *L. ophiocephali* as a new species from *Channa argus* without referring the original description of *L. chinensis* by Yü [72,73]. There are other records of *L. chinensis* from China, Thailand and Korea [74-82]. Both the taxa were synonymised by Sproston et al. [74]. Kim and Choi reported *L. chinensis* as host specific but in the present study, this parasite is obtained from *Clarias batrachus* and is a new host and a geographical record for the parasite [80].

Thus, *Clarias batrachus* (Linn.) acts as potential hosts for various metazoan parasites. Only five species of digeneans, *Allocreadium handiai*, *Orientocreadium batrachoides*, *Emoleptalea protepora*, *Astiotrema reniferum* and a new species, *Phyllodistomum batrachii* is described. Also, three species of cestodes of the family Lytocestidae, *L. indicus*, *L. longicollis* and *L. birmanicus* are redescribed whereas *C. batrachus* acts as a new host and geographical record for an acanthocepalan-juvenile of *Centrorhynchus batrachus* and a copepod *Lamproglena chinensis*.

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