

**ON TWO NEW SPECIES OF THE TREMATODE GENERA, *OPISTHIOPARORCHIS* WANG, 1980
AND *BATRACHOTREMA* DOLLFUS AND WILLIAMS, 1966 (BATRACHOTREMATIDAE),
WITH A REPORT OF A CHINESE SPECIES OF *OPISTHIOPARORCHIS*
FROM ANURAN AMPHIBIAN HOSTS IN INDIA**

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web supplement

ABSTRACT

An examination of small samples of three species of anuran frogs (Amolops afghanus, Rana liebigii and R. khare) originating in Nagaland (northeastern India) revealed three species of trematodes, two of them being new to science. These include Batrachotrema nagalandensis sp. nov. (type host A. afghanus), Opisthioparorchis indica sp. nov. (type host A. afghanus) and O. yunnanse Li, 1996. This is the first record of their respective genera in India. The distinguishing features of B. nagalandensis sp. nov. are - testes located in the middle third of the body, and long cirrus sac extending posteriorly beyond the ventral sucker. O. indica sp. nov. is distinguished by having oral and ventral suckers of almost equal size, intestinal caeca extending beyond the ventral sucker up to the anterior border of testes, the genital pore at the level of the oral sucker and vitellaria confluent medially in the pre and post testicular regions. Kohima (Nagaland) and Rana liebigii form a new locality and new host record, respectively, for O. yunnanse.

KEYWORDS

Amolops afghanus, Batrachotrema nagalandensis sp. nov., India, Nagaland, Opisthioparorchis indica sp. nov., Opisthioparorchis yunnanse, Rana khare, Rana liebigii, Trematode

During an exploratory survey of the helminth parasite fauna of anuran Amphibia in Nagaland (India) several digenetic trematode species were encountered. Two of these forms, on study, were found to be new to science and belong each to the genera *Batrachotrema* Dollfus and Williams, 1966 and *Opisthioparorchis* Wang, 1980. While the former genus has hitherto been reported from Africa (Dollfus & Williams, 1966), China (Wang, 1981; Zhang & Sha, 1985; Liang & Ke, 1988) and Vietnam (Moravec & Sey, 1989) the latter is represented so far in ranid frogs in China. The present communication deals with the description of a new species of each genus, and also forms the first record of the occurrence of these genera in the Indian subcontinent. The occurrence of *O. yunnanse* Li, 1996 is also reported from *Rana liebigii* as a new record.

MATERIALS AND METHODS

Eight specimens (range 1-4) of *Batrachotrema* were collected from the intestine of five *A. afghanus* and only one specimen, from *Rana khare* at Mokokchung in Nagaland. Twenty-one specimens of a form belonging to *Opisthioparorchis* were recovered from the intestine of three *Amolops afghanus* from Mokokchung, the maximum number of this parasite in a single host being 18. Two specimens, also belonging to the same genus but representing another species were collected from one *Rana liebigii* from Kohima.

Whole mount preparations of the flukes were made following standard procedure, using Mayer's carmalum or borax carmine as stains. For scanning electron microscopy (SEM) the specimens fixed in 4% cold neutral buffered formalin were processed and treated with tetramethylsilane as per the method described by Roy and Tandon (1991), metal coated and viewed

under a JSM-35 CF (Jeol) scanning electron microscope at accelerating electron voltage ranging between 10-15kV.

Family Batrachotrematidae

Genus *Batrachotrema* Dollfus and Williams, 1966

***Batrachotrema nagalandensis* sp. nov.**

(Figs. 1-3; Images 1-2^w)

Material examined

Holotype: W 8338/1; from the intestine of *Amolops afghanus* (Günther), Mokokchung, Nagaland (25°-27°4'N & 93°2'-95°15'E). Paratypes: W 8339/1, W 8340/1; from the intestine of *Amolops afghanus* (Günther) and *Rana khare* (Kiyasetuo & Khare), Mokokchung.

Deposited at the Zoological Survey of India, Kolkata.

Etymology

Named after Nagaland state, India.

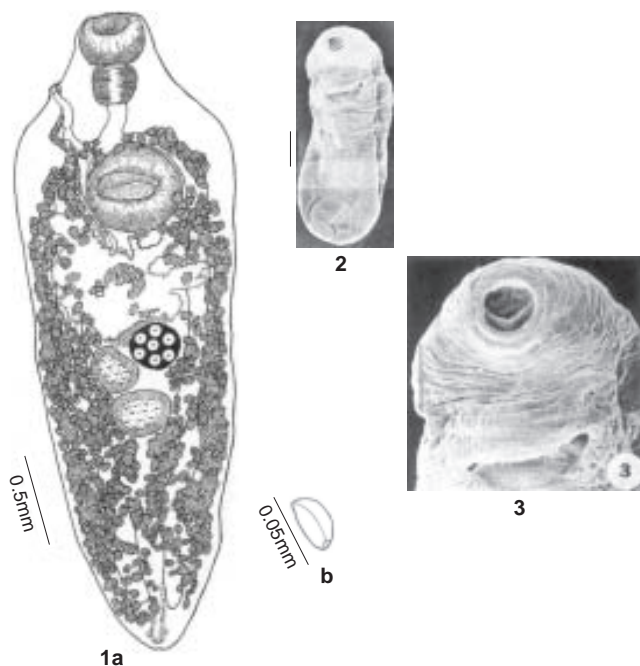
Specific diagnosis

Body unspined, fusiform, testes located in middle third of body; cirrus sac long, extending beyond ventral sucker posteriorly, ovary rounded.

Diagnosis

(Based on measurements of 5 mature specimens and SEM observations on 1 specimen.). Body elongated or some what spindle shaped, broadest at shoulder, tapering and terminating bluntly towards anterior and posterior regions; surface devoid of spines. Oral sucker subterminal. Ventral sucker pre-

^w Also see SEM Images in the web supplement at www.zoosprint.org



Figures 1-3. *Batrachotrema nagalandensis* sp. nov.
1a - Whole mount, dorsal view; 1b - Egg; 2-3 - Scanning electron micrographs: 2 - Full worm, ventral view (scale 100mm); 3 - A magnified view of anterior end, showing the oral end and ventral sucker (scale 100mm).
[See Figures 2 & 3 on the web as Images 1 & 2]

equatorial, located in broader part of body, three times larger than oral sucker, covering half of body in width, strongly muscular. Prepharynx indistinct; pharynx muscular; oesophagus of moderate length or short; intestinal caeca reaching near posterior end. Testes rounded or oval, lying in tandem in middle third of body; anterior testis at level of ovary, slightly smaller than posterior; cirrus sac long, extending posteriorly beyond ventral sucker, lying on its left side, extending anteriorly up to and ending in level with pharynx. Ovary rounded, pretesticular, postacetabular; oviduct, Mehlis' gland complex in region anterior to ovary; uterine coils mainly limited in ovarian and postacetabular area, overlapping intestinal caeca. Genital pore at left side of body, marginal or sub marginal, male and female pores opening into genital atrium. Vitellaria follicular, extending from level of intestinal bifurcation up to posterior most part of body, overlapping intestinal caeca, also scattered in intercaecal fields. Eggs numerous, large, oval. The measurements of this form are given in Table 1.

DISCUSSION

In having a pretesticular ovary, which is nearer to the anterior testis than the ventral sucker and the genital pore lying laterally to the median line in level with the pharynx, the present form bears a close morphological resemblance to the genus *Batrachotrema*. Dollfus and Williams (1966) proposed a new family Batrachotrematidae for the genus. Yamaguti (1971) retained the family as proposed by Dollfus and Williams and Wang (1980) also accepted its validity. However, Prudhoe and Bray (1982), on the basis of its close morphological relationship

Table 1. Measurements (in mm) of *Batrachotrema nagalandensis* sp. nov.

Characters	Range (Mean)	± S.D.
Length of Body	2.38-3.89 (2.93)	0.49
Maximum width of body (at level of ventral sucker)	0.80-1.27 (1.01)	0.202
Oral sucker:		
Length	0.17-0.27 (0.21)	0.043
Breadth	0.26-0.37 (0.33)	0.034
Ventral Sucker:		
Length	0.30-0.49 (0.42)	0.07
Breadth	0.39-0.56 (0.47)	0.07
Pharynx:		
Length	0.17-0.25 (0.22)	0.032
Breadth	0.17-0.31 (0.20)	0.036
Length of Oesophagus	0.24-0.46 (0.41)	0.023
Distance of intestinal caeca from hind end	0.07-0.25 (0.22)	0.037
Intestinal bifurcation from anterior end	0.46-0.75 (0.64)	0.01
Testis I:		
Length	0.20-0.22 (0.22)	0.017
Breadth	0.22-0.34 (0.27)	0.035
Testis II:		
Length	0.24-0.46 (0.31)	0.077
Breadth	0.19-0.36 (0.31)	0.058
Ovary:		
Length	0.22-0.29 (0.26)	0.012
Breadth	0.19-0.28 (0.24)	0.022
Extent of Vitellaria	1.89-3.05 (2.37)	0.419
Eggs	0.03-0.04 x 0.02-0.023 (0.036 x 0.022)	0.002

with the members of *Opecoeliidae*, placed the genus under the latter family.

The genus *Batrachotrema* so far includes five species: *B. petropedetis* Dollfus and Williams, 1966 from *Petropedetes natator* in Sierra Leone, Africa; *B. pseudobagri* Wang, 1981 (from *Pseudobagrus fulvidraconis* and *Pseudogastromyzon zebroidus* in Fujian Province, China); *B. yaanensis* Zhang and Sha, 1985 (from *Rana phrynoides* in Yaan, Sichuan, China); *B. opistosacca* Liang and Ke, 1988 (from *Rana spinosa* in Meixian, Guangdong, China); *B. vietnamensis* Moravec and Sey, 1989 (from *Rana kuhlii* in Hanoi, Vietnam).

A comparison of the present form with the type species reveals several differences between the two. In the present form the testes lie in the middle third of the body and the cirrus sac is long extending posteriorly beyond the ventral sucker. In the type species the testes are located much posteriorly and the claviform cirrus sac is quite small extending posteriorly only up to the anterior margin of the ventral sucker. Both *B. petropedetis* and *B. opistosacca* have an elongated oval body shape and have intestinal caeca extending posteriad a little beyond the testes. In having a fusiform body, the present species resembles *P. pseudobagri*, a species described from piscine hosts, but distinctly differs from it in having a larger body size, a rounded ovary (lobate in *P. pseudobagri*), and a long cirrus sac that extends posteriad much beyond the ventral sucker. In possessing an unspined body, testes situated in the middle third of the body and immediately pretesticular ovary that is

not separated from testes by uterine coils, the present form comes close to *B. petropedetis*, *B. pseudobagri* and *B. opistosacca* and stands apart from *B. yaanensis* and *B. vietnamensis*. In view of the apparent morphological differences and also different amphibian host species the present form is considered a new species under the genus *Batrachotrema*. It is for the first time that a representative of the genus is being reported from the Indian subcontinent.

Family *Batrachotrematidae*

Genus *Opisthioparorchis* Wang, 1980

***Opisthioparorchis indica* sp. nov.**

(Figs. 4-8; Images 3-6^w)

Material examined

Holotype: W 8341/1; from the intestine of *Amolops afghanus* (Günther), Mokokchung, Nagaland (25°-27°4'N & 93°2'-95°15'E)

Paratypes: W 8342/1, 8343/1; same as holotype.

Deposited at the Zoological Survey of India, Kolkata.

Etymology

Named after the country.

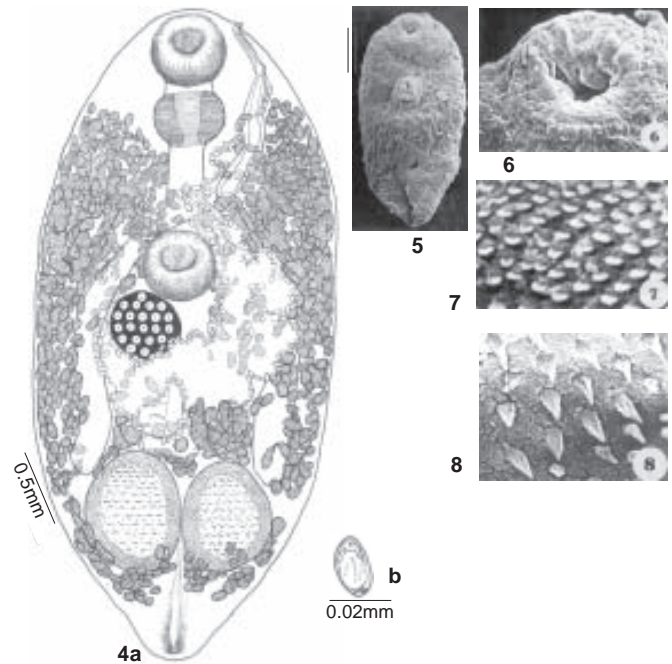
Specific Diagnosis

Intestinal caeca extending much beyond ventral sucker up to anterior level of testes, ventral sucker almost equal to oral sucker in size, genital pore in level with anterior margin of oral sucker, vitellaria extending beyond testes posteriorly, confluent medially in pre- and posttesticular regions.

Diagnosis

(Based on measurements of 10 mature flukes and SEM observations on 2 specimens.). Body elongate or oval with rounded anterior end, rounded or slightly conical posterior end, spinose. Oral sucker subterminal, spherical. Ventral sucker pre-equatorial or equatorial almost equal to oral sucker in size. Prepharynx indistinct; pharynx muscular; oesophagus of moderate length; intestinal caeca extending in postacetabular region up to level of anterior margin of testes. Testes round or oval, lying symmetrically juxtaposed in posterior region of body; cirrus sac well developed, elongated, somewhat S-shaped, recurved, lying on left side of body, extending from near junction of oesophagus and intestinal caeca up to anterior margin of oral sucker. Ovary rounded, pretesticular, postacetabular; receptaculum seminis conspicuous, lying posterior to ovary; uterus pretesticular, occupying major area between ovary and testes, extending anteriorly partially overlapping intestinal caeca. Genital pore marginal on left at level of anterior margin of oral sucker. Vitellaria in varying follicular size, extending in lateral fields from level of pharynx posteriorly up to post-testicular region, becoming confluent medially in intercaecal, pretesticular and post testicular fields. Eggs numerous, ovoid, operculate.

SEM revealed the surface fine topography of the fluke. The body surface is studded throughout with dense spination except for the anterior circum-oral region, which appears devoid of spines. Whereas the spines covering the tegument in most



Figures 4-8. *Opisthioparorchis indica* sp. nov.

4a. Whole mount, ventral view, b. Egg; 5-8. Scanning electron micrographs: 5 - Whole worm, ventral view (scale 100mm); 6 - Oral end, magnified view (scale 10mm); 7 - A magnified view of the tegumental spination in the mid-ventral region (scale 10mm); 8 - Tegument of the posterior part of the body depicting conical spines (scale 10mm)
[See Figures 5-8 on the web as Images 3-6]

parts of the body are scale like with rounded tips, those abounding in the posterior region are conical, with broad base and pointedly tapering distal end. The non-spinous tegument of the anterior region presents a spongy texture. The measurements of this form are given in Table II.

DISCUSSION

In having a well-developed cirrus sac and marginal or submarginal genital pore located in the region of the oral sucker or pharynx, the present form belongs to the family *Batrachotrematidae*.

The genus *Opisthioparorchis* was created by Wang (1980) for an intestinal fluke of *Rana spinosa*, which was characterized by having juxtaposed testes located at the posterior end of the body and intestinal caeca extending up to just near the anterior border of testes. At present the genus includes six species, all described from China: *O. ranae* Wang, 1980 (type species) from *Rana spinosa* in Fujian Province South; *O. pleurogenitus* Wang, 1980 from the same host and locality as those of the type species; *O. boheansis* Wang, 1980 from *Staurois wuyiensis* also from Fujian; *O. megaloonis* Liang and Ke, 1988 and *O. meixianensis* Liang and Ke, 1988, both from *R. spinosa* in Changsha (Meixian, Guangdong Province) and *O. yunnanensis* Li, 1996 also from *R. spinosa* from Yunnan Province.

In having the vitellaria extending in the posttesticular region,

Table 2. Measurements (in mm) of *Opisthioparorchis indica* sp. nov.

Characters	Range (Mean)	± S.D.
Length of Body	1.80-2.33 (2.05)	0.18
Maximum width of body (at level of ventral sucker)	0.89-1.08 (0.97)	0.08
Oral sucker:		
Length	0.21-0.26 (0.24)	0.02
Breadth	0.23-0.30 (0.26)	0.026
Ventral Sucker:		
Length	0.21-0.25 (0.23)	0.014
Breadth	0.21-0.27 (0.24)	0.018
Pharynx:		
Length	0.19-0.23 (0.21)	0.018
Breadth	0.2-0.24 (0.22)	0.016
Length of Oesophagus	0.14-0.23 (0.18)	0.03
Distance of intestinal caeca from hind end	0.58-0.77 (0.68)	0.06
Intestinal bifurcation from anterior end	0.53-0.65 (0.61)	0.04
Testis (right):		
Length	0.33-0.46 (0.39)	0.04
Breadth	0.26-0.33 (0.29)	0.023
Testis (left):		
Length	0.33-0.41 (0.38)	0.03
Breadth	0.25-0.32 (0.28)	0.029
Cirrus sac Length	0.52-0.66 (0.57)	0.05
Ovary:		
Length	0.17-0.25 (0.22)	0.027
Breadth	0.17-0.23 (0.20)	0.022
Receptaculum seminis:		
Length	0.36-0.53 (0.44)	0.07
Breadth	0.03-0.065 (0.047)	0.013
Eggs	0.027-0.037 x 0.011-0.018 (0.031 x 0.015)	0.002

the present form resembles the type and other species but stands apart from *O. pleurogenitus* and *O. yunnanse*, in both of which the vitelline follicles are distributed only in the pretesticular region and the testes are the posterior most structures in location in the fluke body. It also differs from all those species having posttesticular vitellaria in several characters; the latter species, have a much smaller body size, the ventral sucker is smaller than the oral and unlike the present form, the vitellaria do not become confluent medially in the pretesticular, inter-intestinal and posttesticular regions.

In view of the conspicuous differences of the present form with the hitherto known species of *Opisthioparorchis*, it is proposed to consider this as a new species named *O. indica*.

***Opisthioparorchis yunnanse* Li, 1996**
(Fig. 9)

Material examined

From the intestine of *Rana liebigii* (Günther), Kohima (Nagaland, 25°-27°4'N, India).

Diagnosis

(Whole mount). Body elongate, somewhat narrow anteriorly, broader posteriorly, with bluntly rounded ends, spinose. Oral sucker subterminal, almost twice as large as ventral sucker,



Figure 9. *Opisthioparorchis yunnanse* Li, 1996
Whole mount, ventral view

Table 3. Measurements (in mm) of *Opisthioparorchis yunnanse*

Characters	Measurement
Length of Body	2.5
Maximum width of body (at level of ventral sucker)	1.94
Oral sucker:	
Length	0.28
Breadth	0.29
Ventral Sucker:	
Length	0.08
Breadth	0.08
Pharynx:	
Length	0.18
Breadth	0.20
Length of Oesophagus	0.09
Distance of intestinal caeca from hind end	0.58
Intestinal bifurcation from anterior end	0.6
Testis (right):	
Length	0.37
Breadth	0.35
Testis (left):	
Length	0.38
Breadth	0.31
Cirrus sac Length	0.97
Ovary:	
Length	0.23
Breadth	0.23
Receptaculum seminis:LengthBreadth	0.420.047
Eggs	0.041 x 0.021

latter equatorial in position. Prepharynx short, pharynx muscular; oesophagus short; intestinal caeca extending posterior up to a little in front of testes. Testes round or oval, juxtaposed near posterior extremity of body; cirrus sac club-shaped, elongated, extending from a little in front of ventral

sucker anteriorly up to level of oral sucker. Ovary rounded, just by side or slightly posterior to ventral sucker; uterus mainly in near post ovarian region, extending as ascending narrow tube anteriorly. Genital pore marginal, on left at level of oral sucker. Vitellaria extending extensively from level of oesophagus posteriorly up to just in front of testes. Eggs elliptical, operculate. The measurements of this form are given in Table III.

REMARKS

The whole mount preparation of one specimen collected from *Rana liebigii*, though apparently resembled *Opisthioparorchis* species, turned out to be different from the new species of the genus described above. In its general morphology (elongated body, oral sucker larger than the ventral sucker- almost double the size, vitellaria limited to only pretesticular zone and long club-shaped cirrus sac), the present specimens stands close to *O. yunnanse* Li, 1996, which was originally described from *Rana spinosa* from Yunnan Province, China. However, the only conspicuous deviation it shows from this species is the length of the oesophagus; in *O. yunnanse* the oesophagus is quite long, though short in the present form. However, considering that just one character i.e. length of the oesophagus, is not enough to erect a new species, the present form is considered representing *O. yunnanse*.

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ANT DIVERSITY IN SOME SELECTED LOCALITIES OF SATTUR TALUK, VIRUDHUNAGAR DISTRICT, TAMIL NADU

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Social insects in general, and ants in particular have achieved unprecedented ecological success and dominance in tropical ecosystem (Gadagkar *et al.*, 1993). Social way of life adapted by all ant species brings with it both benefits and cost (Brain, 1975). Being such dominant and successful component of ecosystems, it is likely that ants would turn out to be good indicators of the well being of the ecosystem (Daniels, 1991). However, a literature survey revealed that only limited studies are available on diversity of ants. This may be due to lack of appropriate methodologies of tropical insect communities, especially ants (Gadagkar *et al.*, 1990). The present study was carried out in an attempt to understand and measure the status of ant species diversity in some selected localities of Sattur taluk (9°10'N & 77°52'E; 61.19m), Virudhunagar district, Tamil Nadu, in riverine, cultivated and industrial areas.

The study was carried out for seven months from August 2001 to February 2002. The ants were collected from three selected localities: cultivated areas (paddy, cotton, brinjal, ladies finger, *Cassia fistula*, banana, coconut, guava, palm etc), riverine areas (vegetation found along the banks of Arjuna and Vaiparu rivers), and industrial areas (match factories, fire works and printing industries). The average rainfall during the period was 75.84mm in Sattur taluk. Maximum and minimum temperatures were 35°C and 26°C, respectively. All the collected specimens were preserved in 70% alcohol. Ants were separated and sent to the Center for Ecological Science at Indian Institute of Science, Bangalore for identification and confirmation of ant specimens, with the help of Dr. R. Gadagkar, Director, CES. Relative abundance of ant species were calculated using the following formula (Micheal, 1986).

$$\text{Relative abundance} = \frac{\text{Total number of individuals of the species}}{\text{Total number of individuals of all species}} \times 100$$

A total of 25 species of ants belonging to 14 genera distributed in six subfamilies were recorded (Table 1). The subfamilies included Formicinae (9 species) followed by Myrmicinae (8 species), Pseudomyrmicinae (4 species), Ponerinae (2 species), Dorylinae / Dolichoderinae (1 species). The study revealed that more number of ant species were recorded in the riverine (24 species) and cultivated areas (20 species).