# ON SOME CILIATE PARASITES OF FROGS AND TOADS OF KARNATAK, BOMBAY PRESIDENCY\*

By J. C. Uttangi, Department of Zoology, Karnatak College, Dharwar.

### CONTENTS.

						PAGE.
Introduction	••	• •	• "	• •	• •	139
Notes on Hosts	••	• •	• •	• •	••	140
Systematics	••	• •	••	• •	••	140
Summary	••	••	• •	• •	••	155
References	••	• •	• •	• •	••	155

#### Introduction.

The occurrence of binucleate opalinids in Indian Anura has been a subject of great controversy in the past. Bezzenberger (1904), for the first time, described Zelleriella macronucleata (Bezz.) from an Asian toad, Bufo melanostictus Schneider, but his findings were later rendered doubtful by Metcalf (1923) who opened thirty-nine specimens of this toad to find no Zelleriella in them. Bhatia and Gulati (1927) who conducted extensive survey of the ciliate parasites of the Anura of the Punjab also reported a complete absence of both Zelleriella and Protoopalina in Bufo melanostictus as well as other Anurans they studied. The absence of these ciliates in Indian Anura was, thus, more or less accepted, until Nie (1935) again reported Protoopalina caudata microhyla Nie, from an Indian frog Microhyla ornata Dumeril and Bibron (Ind. Mus. Reg. No. 17287), a specimen of which collected at Harnai, Ratnagiri District, Bombay, was supplied to him by the Indian Museum, Calcutta (Metcalf, 1940; p. 472). This discovery of Nie evidently raised the question asked by Metcalf (p. 577) "Why are there no more Protoopainas in these Indian Ocean Lands?", and, thus, led the writer to undertake this interesting piece of work.

The present investigation establishes, beyond doubt, the existence of binucleate opalinids in the Anura of Karnatak, and includes the description of five new forms, i.e., Protoopalina indica, P. karnatakensis, P. dharwarensis, Zelleriella microhylae and Z. froilanoi. Since the locality is situated in South India which forms the most ancient part of the Asian continent, the presence of Zelleriella and Protoopalina in this region, may modify the present conclusions in the field of Zoogeography.

During the course of this work, the following twelve species of other ciliates found parasitic in the gut of the hosts examined have also been recorded: Nyctotherus macropharyngeus Bezz., N. magnus Bezz., N. magnus malabarica De Mello., N.cordiformis Stein., Balantidium helenae Bezz., B. duodeni Stein., B. gracile Bezz., Opalina ranarum Purk et Val., Cepedea virgula (Dobell) Metcalf., C. philauti sp. nov., and C. dimidiata

<sup>\*</sup> Thesis submitted for the degree of M.Sc. of the University of Bombay.

naglavensis subsp. nov. Most of the host species have been examined for the first time in India, and in the cases of those already studied more ciliates have been added to the list of their parasites.

The smears made on cover-glasses were either fixed in Schaudinn's sublimate alcohol and stained with Mayer's haemalum or they were fixed in Bouin's fluid and stained with Iron-haematoxylin. Some smears stained with Borax-carmine were also found to be very useful.

All drawings are made with the aid of camera lucida.

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### Notes on Hosts.

1. Bufo melanostictus Schneider.—The toad is very common in Dharwar and on account of its sluggishness, is easy to collect. Out of twenty-one specimens each measuring about 80 mm. from snout to vent, only seven were found infected.

Habitat: Gardens. Collected during December, 1944.

Ciliates recorded for the first time: Nyctotherus macropharyngeus Bezz.

\*2. Micricalus opisthorhodus Boulenger.—On account of its rarity, only five specimens each measuring about 63 mm. from snout to vent could be examined. All the five were well infected with parasites.

Habitat: Tank near Dharwar. Collected during October, 1944. Ciliates found: Nyctotherus macropharyngeus Bezz., N magnus Bezz., Balantidium helenae Bezz., B. duodeni Stein., B. gracile Bezz., Opalina ranarum Purk. et Val.

\*3. M. saxicola Boulenger.—This is also a very rare frog. Only two specimens each measuring 40 mm. from snout to vent were obtained and examined. Both were found to be well infected with parasites.

Habitat: Tank near Dharwar. Collected during October, 1944. Ciliates found: Nyctotherus macropharyngeus Bezz., N. magnus Bezz., N. magnus Bezz., N. magnus malabarica De Mello., N. cordiformis Stein., Balantidium helenae Bezz., B. duodeni Stein., B. gracile Bezz., Opalina ranarum Purk et Val., and O. coracoidea Bezz.

\*4. Microhyla ornata Dumeril and Bibron.—This frog occurs in large numbers round about Dharwar. The young ones were generally found in day time in paddy fields during rains; but the adults due to their nocturnal and burrowing habits are rarely found during the day and usually leave their abode after sunset when large number of them may be met with. The best time to collect the young ones is during September and November. As many as 25 tadpoles and 10 froglings collected at Naglavi, a forest area about twelve miles from Dharwar, showed heavy infections of both Zelleriella and Protoopalina.

Habitat: Paddy fields. Collected during October, 1944 and August 1945.

<sup>\*</sup> The host species preceded by an asterisk(\*) have been examined for their ciliate parasites for the first time in India.

Ciliates found: Nyctotherus cordiformis Stein., Protoopalina indica n. sp. P. karnatakensis n. sp., P. dharwarensis n. sp. and Zelleriella microhylae n. sp.

\*5. Philautus sp.—As stated by McCann (1932), it is one of the most elusive frogs and is rare and difficult to locate. As only young ones were obtained the species could not be ascertained. Some specimens were infected with Zelleriella. Fifteen juvenile frogs each about 16 mm. long and five tadpoles each measuring about 48 mm. long were examined. All the five tadpoles and ten out of the fifteen froglings were found with parasites.

Habitat: Paddy fields at Naglavi. Collected during September, 1944 and August, 1945.

Ciliates found: Nyctotherus orcdiformis Stein., Cepedea virgula (Dobell)., Zelleriella froilanoi n.sp., and Cepedea philauti n.sp.

6. Rana cyanophlyctis Schneider.—The frog is most common in Dharwar. Out of thirteen specimens (each measuring about 43 mm. long from snout to vent) ten were found infected.

Habitat: Ponds. Collected during April, 1944.

Ciliates recorded for the first time: Nyctotherus magnus Bezz., N magnus malabarica De Mello., Balantidium gracile Bezz., Cepedea dimidiata naglavensis n. sp.

\*7. R. leptodactyla Boulenger.—The species is somewhat rare. Two specimens each measuring 50 mm. from snout to vent, were studied. Habitat: Ponds. Collected during October, 1944.

Ciliates found: Nyctotherus macropharyngeus Bezz., N. cordiformis Stein., Balantidium helenae Bezz., and B. duodeni Stein.

8. R. limnocharis Wiegman.—Five specimens of this common frog, each measuring about 50 mm. long, were examined. All of them were found to be parasitized.

Habitat: Ponds. Collected during April, 1944.

Ciliates recorded for the first time: Nyctotherus magnus malabarica De Mello., N. cordiformis Stein., Balantidium duodeni Stein, B. gracile Bezz., Opalina ranarum Purk et Val and O. coracoidea Bezz.

\*9. R. temporalis Boulenger.—Only two specimens of this rare frog each 55 mm. long were examined.

Habitat: Ponds. Collected during October, 1944.

Ciliates found: Nyctotherus macropharyngeus Bezz., N. magnus malabarica De Mello., N. cordiformis Stein., Balantidium helenae Bezz., B. duodeni Stein., B. gracile Bezz., Opalina ranarum Purk et Val., and O. coracoidea Bezz.

### SYSTEMATICS.

# Nyctotherus macropharyngeus Bezz.

This ciliate is very common in the frogs of Dharwar. Besides being present in the old hosts (Rana limnocharis Wieg. and Rana cyanophlyctis Schn.), the parasite was met with in a number of new hosts: Micrixalus saxicola Boul., Micrixalus opisthorhodus Boul., Rana temporalis Boul., Rana leptodactyla Boul., and Bufo melanostictus Schn. In M. saxicola, the form was slightly more ellipsoidal than oval, about two times as long as broad; the cytoplasm appeared very much alveolated; the contractile

vacuole situated at the posterior end was single; the macronucleus usually surrounded by a halo varied in its shape; the micronucleus, however, could not be detected. In other hosts, typical forms were found.

Measurements (in microns) of N. macropharyngeus Bezz.

	No. of	Body: length × b	Body (length:	Average		
Name of Host.	parasites studied.	Range.	Average.	breadth) average.	nucleus.	
Micrizalus saxicola—						
Host I	91	200—350×110—180	275×135	2.03:1	$55 \times 31$	
Host II	62	172—335×105—190	253×147	1.75:1	$50 \times 26$	
Micrixalus opisthorhodus	32	200-305 × 110-160	252×135	1.80:1	48×28	
Rana leptodactyla	25	220—290×115—150	255×132	1.93:1	43×25	
Rana temporalis	67	205—305×105—150	255×127	2.00:1	46×27	
Bufo melanostictus*	11	200—255×120—150	227×135	1.66:1	55×30	

<sup>\*</sup>Parasites scanty in number.

### Nyctotherus cordiformis Stein.

With the exception of Rana temporalis and R. leptodactyla, the parasite, though scanty in number, was found in all the hosts examined. In Bufo melanostictus, the ciliate reached a maximum body size of  $138 \times 88$  microns, and appeared somewhat beanshaped. Those observed by Bhatia and Gulati (1927), in the same species of the host, were smaller. The cytopharynx in many individuals was not as much developed and curved as in the typical form described by Stein (1867). In M. saxicola and M. opisthorhodus, individuals having a somewhat longer cytopharynx were met with. De Mello (1932) observed a similar elongation of the cytopharynx in the forms studied by him from Rana malabarica. The macronucleus also showed variations in the shape and size from host to host.

Measurements (in microns) of Nyctotherus cordiformis Stein.

	No. of	Body: length ×	Body (length:	Average	
Name of Host.	parasites studied.	Range.	Average.	breadth) average.	nucleus.
Micrixalus saxicola	17	70—110×50—70	90×60	1.50:1	44×9
" opisthorhodus	10	70—100 × 40—60	85×50	1.70:1	45×15
Rana cyanophyctis	18	90—130×50—80	103×60	1.71:1	55×15
Rana limnocharis—					
Host I	20	110—150 × 80—105	130 × 92	1.41:1	50×15
Host II ,.	12	75—120 × 45—75	97×60	1.61:1	50×15
Mirrohyla ornata	8	85—125 × 65—85	105×75	1.40:1	50×25
Philautus sp	5	115-140×60-80	127×70	1.81:1	55 × 30
Bufo melanostictus	14	110—155×65—90	132×77	1.71:1	55 × 30

### Nyctotherus magnus Bezz.

This giant Nyctotherus was met with only in four hosts, namely, Rana limnocharis, R. temporalis, Micrixalus saxicola and Rana cyanophlyctis of which the first three are new hosts for this ciliate. In all these infections the individuals were rather few in number. Their exceptionally large body measurements helped to distinguish them from other species such as N. macropharyngeus Bezz., N. cordiformis Stein. which were always found associated with it. The form though small in size as compared to Bezzenberger's approached the typical in character. However, the cytoplasm in some individuals exhibited a much alveolated condition, and showed large food-vacuoles in which were found ingested specimens of Balantidium helenae. A similar instance was noted by Carini (1939) in Nyctotherus vorax Carini., in the body of which he found few specimens of N. cordiformis being ingested and digested there as food. Whether such food-habits are normal or the smaller ciliates fall as prey by chance during the process of feeding cannot definitely be stated here.

Measurements (in microns) for Nyctotherus magnus Bezz.

Name of Host.		No. of	Body: length × b	Body		
		No. of parasites studied.	Range.	Average.	(length: bredth) average.	Average nucleus.
Rana cyanophlyctis	••	5	550—610 × 335—380	580 × 357	1.34:1	180×50
Rana limnocharis	••	1	485 × 340		1.42:1	180×45
Rana temporalis	••	4	340518 × 230340	429×285	1.50:1	186 × 60
Micrixalus saxicola*		1	620 × 450		1.37:1	200×60

<sup>\*</sup> Showed cannibalistic character.

# Nyctotherus magnus malabarica De Mello.

Typical forms were observed in Rana limnocharis, Rana temporalis, R. leptodactyla and Micrixalus saxicola, all of which have been opened for this parasite for the first time. Individuals from Rana leptodactyla and R. temporalis showed considerable variations in the curvature of their cytopharynx. The inner end was not so bent as shown by De Mello (1932). The furrow running from the tip of the cytopharynx to the anal opening was very prominent. The finely granulated cytoplasm appeared somewhat thin and less alveolated. The micronucleus could not be traced throughout. In Micrixalus saxicola, the forms though small in size, were typical in character.

Measurements (in microns) for Nyctotherus magnus malabarica De Mello.

Name of H	No. of	Body: length × 1	Body (length:	Average		
Name of Host.	parasites studied.	Range.	Average.	breadth) average.	nucleus.	
Micrixalus opisthorhodus	6	120—150 × 90—110	135×100	2.00:1	55×20	
Rana temporalis	10	135—190 × 90—130	162×110	1.56:1	$50 \times 25$	
Rana leptodactyla—						
Host I	21	140—200 × 95—145	170×120	1.41:1	$55 \times 30$	
Host II	25	200—280×110—170	240×140	1.91:1	$55 \times 30$	
Rana limnocharis	17	105—170×75—110	137×92	1.48:1	$60 \times 30$	

### Balantidium helenae Bezz.

Though Microhyla ornata, Philautus sp., and Bufo melanostictus were entirely devoid of this ciliate, it was abundantly present in other host species. In Micrixalus saxicola, the form varied from elongately oval to broadly oval. The position of the macronucleus varied in many individuals. Out of 75 specimens 32 had their nucleus in about the middle of the body, while in the remaining 43, it was found to lie in the posterior half of the body. The cytoplasm contained some irregular bodies which were stained like the nucleus. The anal opening situated at the posterior pole was clearly visible. The boring apparatus as reported by Ray, (1932) and Chakravorti (1933) was not detected. In M. opisthorhodus, Rana temporalis, R. leptodactyla, R. limnocharis, and R. cyanophlyctis typical forms were found.

The broadly oval forms have been described under a new species B. ovale (Dobell 1910, p. 74). After studying these forms in the Lahore frogs, Bhatia and Gulati (1927, p. 107) came to the conclusion that they were identical in structure with B. helenae and differed only in size; and should, therefore, be treated as one and the same species. Similar forms having been met with in the hosts of Dharwar, the writer is of the opinion that Bhatia and Gulati are probably correct.

Measurements (in microns) for Balantidium helenae Bezz.

Name of Host.		No. of	Body: length ×	breadth	Body (length:	Average nucleus.	
		parasites studied.	Range.	Average.	breadth) average.		
Micrixalus saxicola		75	80-140 × 40-150	110×95	1.10:1	30×12	
M. opisthorhodus		10	$45\text{-}60 \times 28\text{-}32$	$52 \times 30$	1.73:1	$20 \times 10$	
Rana temporalis		22	55-115 × 30-50	$85 \times 40$	2.12:1	30×10	
R. leptodactyla		34	50-105 × 30-65	$77 \times 47$	1.50:1	$25 \times 10$	
R. limnocharis		20	$40 \text{-} 90 \times 30 \text{-} 45$	$65 \times 37$	1.71:1	$30 \times 9$	
R. cyanophlyctis		49	$50\text{-}85 \times 25\text{-}50$	$67 \times 37$	1.81:1	30 × 10	

#### Balantidium gracile Bezz.

With the exception of *Microhyla ornata*, *Bufo melanostictus* and *Philautus sp.* the parasite occurred in all the hosts examined at Dharwar. It was usually located in the intestine. Bezzenberger (1904) found the macronucleus lying mostly at the hinder end and seldom in the middle. In Dharwar forms it was usually in the middle.

Measurements (	in microns)	for	Balantidium	gracile	Bezz.
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Name of Host.		No. of	Body: length ×	Body (length:	Average	
		parasites studied.	Range.	Average.	breadth) average.	nucleus.
Micrixalus saxicola*		27	145-210 × 25-30	177×27	6.55: 1	20×10
M. opisthorhodus		46	$95\text{-}185 \times 20\text{-}35$	$140 \times 27$	5.18:1	$20 \times 9$
Rana temporalis†		4	$130\text{-}155 \times 20\text{-}35$	$142\times25$	5.60:1	20×11
Rana leptodactyla		44	$170 \text{-} 285 \times 20 \text{-} 35$	$227 \times 27$	8.40:1	$36 \times 10$
Rana limnocharis		65	90-195 × 30-40	142  imes 35	4.05:1	20×10
Rana cyanophlyctis		24	120-260 × 20-25	$190\times 22$	8.60:1	30 × 10

<sup>\*</sup> Pure culture in small intestine.

#### Balantidium duodeni Stein.

The ciliate occurred in the new hosts, Rana temporalis, R. leptodactyla, Micrixalus saxicola, and M. opisthorhodus as well as in the old hosts R. limnocharis and R. cyanophlyctis. Typical specimens were met with in all the hosts.

Comparing the characters of *B. rotundum* Bezz. with *B. duodeni* Stein, it is found that the former differs from the latter in having a straight dorsal surface (which, perhaps, is due to the much rounded sides) that gives the parasite an appearance of an egg. In other characters like the nucleus, the triangular area in the cytoplasm and the peristome they are quite identical. In the specimens studied at Dharwar, it was noted that both oval and egg-shaped forms were present. Those that were small in size appeared egg-shaped, while the larger forms appeared longer and oval. *B. rotundum*, therefore, may well be compared to these smaller forms of *B. duodeni*. And since *B. rotundum* is not structurally very different from *B. duodeni*, I am inclined to think that both these duodenal forms are identical.

Measurements (in microns) for Balantidium doudeni Stein.

	No. of	Body: length ×	breadth	Body (length:	Average	
Name of Host.	parasites studied. Range.		Average.	breadth) average.	nucleus.	
Micrixalus saxicola*	19	25-43×14-30	$34 \times 22$	1.54:1	10×7	
M. opisthorhodus	53	$30-55 \times 20-30$	42×25	1.68:1	11×8	
Rana temporalis	23	$30\text{-}48 \times 20\text{-}24$	39×22	1.77:1	10×8	
R. limnocharis†	7	40-50 × 20-30	45×25	1.80:1	9·5×9	
R. cyanophlyctis	16	$35-45 \times 25-30$	40×27	1.58:1	10×8	

<sup>\*</sup> Abundant in duodenum.

<sup>†</sup> Parasites scanty in number.

<sup>†</sup> Parasites scanty in number.

# Opalina ranarum Purk and Val.

The parasite occurred both in the new hosts (Rana temporalis, Micrixalus saxicola and M. opisthorhodus) and in the old ones (Rana limnocharis and Bufo melanostictus). In all these infections the parasites were abundant and were usually lodged at the anterior end of the rectum. In the same infection, both oval and truncated forms were noticed. Metcalf (1923) has considered these forms as two separate subspecies. Since all these forms are common in all the infections studied at Dharwar, it seems that they are only the variations, of one and the same species. The parasite resembled the typical form in all other characters.

Measurements	(in	microns)	for	Opali	na	ranarum	Purk	k de	Val	l.
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		No. of	Body: $length \times$	Body	Average		
Name of Host.	Name of Host.		Range.	Average.	(length: breadth) average.	nucleus (diameter).	
Micrixalus saxicola*		21	$170-210 \times 75-200$	190×137	1.84:1	3-4	
M. opisthorhodus		61	$225\text{-}410 \times 110\text{-}185$	317×147	2.13:1	34	
Rana temporalis		44	$170\text{-}280 \times 70\text{-}175$	225×122	1.84:1	3—4	
R. limnocharis		21	$130 \text{-} 190 \times 70 \text{-} 90$	160×80	2.00:1	3.5-4	
Bufo melanosticutus		31	$110 - 180 \times 55 - 100$	147×77	1.90:1	3—4	

<sup>\*</sup> Truncated forms.

### Opalina coracoideo Bezz.

In Dharwar, the species occurred in three hosts, Rana limnocharis. R. temporalis and Micrexalus saxicola. Of these the latter two are recorded here as new hosts for this parasite. The ciliate showed a number of shapes especially in the length of the beak which varied from individual to individual. In some, the beak was considerably reduced while in others it was exaggerated into a well developed tail. Bhatia and Gulati (1927) have described these forms with elongated beaks under a new species, Opalina coracoidea lahorensis. Metcalf (1940) while studying three infections of O. coracoidea from R. cyanophlyctis of Bogawan talava, Ceylon, found the beak to be either long or short or absent. These differences in the length of the beak were also present in the infections studied at Dharwar.

Measurements (in microns) for Opalina coracoidea Bezz.

Name of Host.		No. of	Body: length $\times$ b	readth	Body (length:	Average nucleus (diameter).	
		parasites studied.	Range.	Average.	breadth) avcrage.		
Micrixalus saxicola				_			
Host I*		20	$190-270 \times 100-250$	230×175	1.31:1	45	
Host II†	}	27	$150\text{-}240\times90\text{-}155$	195×122	1.51:1	4	
Rana temporalis‡		31	$125\text{-}325\times70\text{-}200$	225×135	1.65:1	4	
R. limnocharis§		12	130-210 × 75-120	170×97	1.74 :1	3-4	

<sup>Length of beak (average) 30-40.
Length of beak (average) 30-40.
Length of beak (average) 30-42.
Length of beak (average) 30-40</sup> 

### Cepedea virgula (Dobell) Metcalf.

The ciliate was met with in *Philautus sp.* which was heavily infected. The horizontally placed endospherules were well stained with iron-haematoxylin.

Measurements in microns.—35 parasites.

Body (length  $\times$  breadth): range, 80-125 $\times$ 35-50; average, 102 $\times$ 42; length: breadth (average), 2.33:1; average nucleus (diameter), 5.

### Cepedea philauti, sp. nov.

The body, clothed with short cilia, is cylindrical and somewhat fusiform with the posterior middle of the body having the greatest width. The anterior and the posterior ends are broadly rounded. The coarsely alveolated cytoplasm is not differentiated into ectosarc and endosarc and appears somewhat compact near the anterior pole. The features that are characteristic in this new species are the numerous large and oval nuclei spread irregularly in the cytoplasm. Incomplete transverse constrictions similar to those seen in *C. formosae* Met. were observed in some individuals.

Host.—Philautus sp.

Measurements in microns.—19 individuals.

Body (length  $\times$  breadth): range, 255-360  $\times$  50-80; average, 307  $\times$  65; Average nucleus (diameter), 4-5. Dividing nucleus (oval),  $10 \times 7$ . Cilia line interval, anterior region 1, posterior region, 2. Diameter of endospherule, 3-4.

Key to the species of Cepedea.

	I	J	
I (3	) Anterior end with a spine-like projectio	n	2
2	Body triangular in cross-section. Po		
	bluntly rounded. Length, 82 µ	• •	C. punjabensis Bh. & G.
3 (1	) Anterior end without a spine-like project	tion	4
4 (15	) Body greatly elongated	••	5
5 (10)	Body with transverse constrictions	• •	6
6 (9)	With one or two incomplete constriction	ns	7
7 (8	) Spindle-shaped body with elliptical nuc	clei.Length,	
	$170 \mu$	••	C. formosae Met.
8 (7	) Fusiform body with spherical nuclei. Le	ngth, 307 μ	C. philauti sp. n.
9 (6)	With many complete constrictions. Leng	gth, 365 µ	C. segmentata. Met.
10 (5)	Body without any constrictions		11
11 (12)	Nuclei ellipsoidal. Length, 680-1000 µ	••	C. longa Bezz.
12 (11)	Nuclei spherical	• •	13
13 (14)	Cilia short. Length, 848	• •	C. dolicosoma Met.
14 (13)	Cilia long. Length, 500 µ		C. ophis Met.
15 (4)	Body not greatly elongated	•	16
• •	Body cylindrical	••	17
	Sides of the body curved, anterior end ro	ounded and	
- ( )	posterior tapering to a point	• •	18
18 (19)	With a naked spine at the posterior pole s	mall round	
	nuclei (diameter) 3.8 $\mu$ , Length, 124 $\mu$	• •	C. spinifera Met.
19 (18)	Without a naked spine at the posterior po	ole	20
20 (23)	Nuclei spherical	• •	21
21 (22)	Endospherules round and irregularly	placed	<b>~</b>
	Length, $180 \mu$		C. dimidiata Stein.
22 (21)	Endospherules elongated and transversel	y placed	C mingula (Dahall)
	Length, $98 \mu$	• 1	C. virgula (Dobell)
			9

C. sialkoti Bh. & G.

sp. nov.

23 (20) Nuclei dumbell-shaped. Length, 131 μ
24 (17) Sides of the body straight. Anterior end presenting a vacuolated appearance. osterior end rounded, C. celebensis Met. sometimes pointed. Length, 125-440 µ C. thiagi De Mello. 25 (16) Body lanceolate. Anterior end broad and rounded. Posterior, slender and tapering to a point. Nuclei only 4 or 5, Length, 82  $\mu$  ... 26 (25) Body obovate. Length, 315  $\mu$ C. lanceolata Met. C. obovoidea Met. 170 μ C. globosa Met. 29 (28) Nuclei spherical. Cilia sparse and short. Length, C. bandivii Met. 87 µ 30 (27) Body sub-cylindrical 31 (32) Both ends rounded or anterior end less pointed than the posterior. Length,  $35-250 \mu$ C. subcylindrica De Mello.

# Cepedea dimidiata naglavensis subsp. nov.

Description and discussion.—

32 (31) Both ends pointed. Length,  $64-89 \mu$ 

Cepedea dimidiata has a number subspecies. They are generally described as slender, more slender, stocky and Zelleriella forms. De Mello (1944) found more slender forms in the present host (R. cyanophlyctis) from Nova Goa, and named them as C. dimidiata cottoniana. The forms found in R. cyanophlyctis from Dharwar differ from cottoniana in having a much attenuated posterior end, and also in having a greater number of nuclei. The present sub-species is named as Cepedea dimidiata var. naglavensis after the locality from where the host was collected.

Host.—Rana cyanophlyctis

Measurements in microns.—28 parasites.

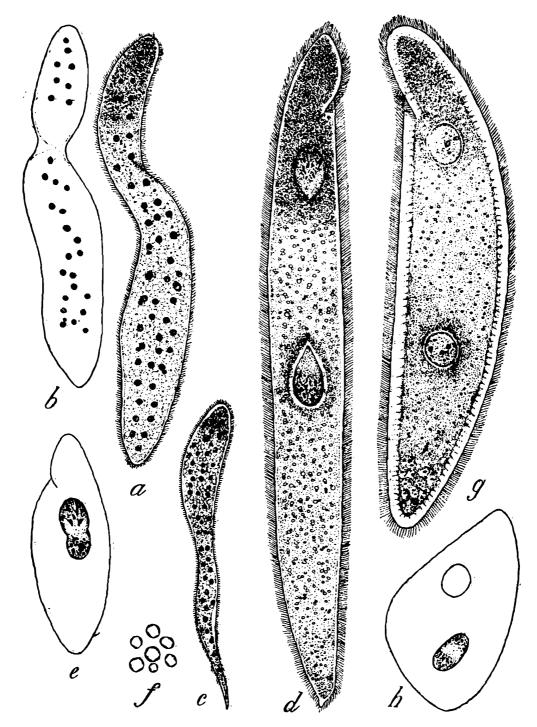
Body (length  $\times$  breadth): range,  $180-255 \times 15-30$ ; average  $207 \times 22$ , length: breadth (average), 9.40:1. Average nucleus (diameter): 3-4.

# Key to subspecies of C. dimidiata

# Protoopalina indica, sp. nov.

Description.—The body is cylindrical, broadest near the anterior end and gradually tapering towards the posterior. The anterior pole is bent to one side while the posterior narrowing into a rounded point appears somewhat mamillionated. The cytoplasm is not well diffentiated into cortical and medullary zones. Numerous large oval endospherules (stained well with iron-haematoxylin) were seen in the medullary zone. Towards the anterior pole these endo-spherules aggregate, so

that, a more compact and denser region is formed. It is somewhere in this compact region that the anterior nucleus is usually located while the posterior nucleus is almost always in the middle of the body. The two pear-shaped nuclei are so placed that they lie with their pointed ends facing one another. The so called nuclear thread (consisting of attenuated nuclear membrane) connecting the two nuclei was not



Text-fig. 1. a and b.—Cepedea philauti, sp.nov. a. Normal individual showing general structure:  $\times$  215; b. Dividing individual:  $\times$  215: c. Cepedea dimidata naglavensis, sub sp. nov.:  $\times$  430. d-e.-Protoopalina indica sp. nov. d. Normal individual showing, general structure:  $\times$  430; e. Daughter individual from a recent division; f. Endospherules under oil-immersion objective; g & h.—Protoopalina karnatakensis, sp. nov. g. Full grown slender individual showing general structure:  $\times$  430; h.—A stocky (Zelleriella) form in division:  $\times$  430.

visible. The chromatin element of the nucleus in the normal individual is broken up into numerous bits (the nucleoli) the exact number of which could not be determined. In some the number was more than

twenty. The cilia were long and thick. Individuals undergoing division (both transverse and longitudinal), were present. The daughter cells come from transverse division were all uninucleated.

# Host.—Microhyla ornata

Measurements in microns.—61 individuals.

Body (length  $\times$  breadth): range, 135-415  $\times$  40-75; average (L $\times$ B) 275  $\times$  57; length: breadth (average) 4.82:1; nucleus (length  $\times$  breadth): range, 27-33  $\times$  14-17. Endospherule (length  $\times$  breadth): range, 2.5-3.5 $\times$ 1.5-2. Cilia line interval, anterior region, 1.8; middle region 2.10; posterior region, 3.00. Width of ectosarc, 3.00.

Comparison.—On account of its slender pointed posterior end, as well as the body measurements and the shape of the nucleus, *P. indica* is classed under the sub-generic group II (Metcalf 1940, p. 571-576).

The following table shows the specific differences of the group.

# Key to subgeneric group II.

	ney to subjeneric group i	1.
1	(2) Posterior pole tapering to a sharp point and sometimes	
	with a posterior process	3
3	(6) Posterior end broad and abruptly ending in a point	4
4	(5) Posterior end not curved. Nucleoli 6 in number Nucleus (L $\times$ B) 40 $\times$ 26 $\mu$ . Body (L $\times$ B) 200 $\times$ 95	
	$\mu$	P. caudata (Stein).
	(4) Posterior end curved. Tail large and blunt. Nucleoli 8 in number. Nucleus (L $\times$ B) 30 $\times$ 14 $\mu$ . Body (L $\times$ B) 90 $\times$ 74 $\mu$	P. macrocaudata. Met.
6	(3) Posterior end elongated and gradually tapering to a narrow point	7
7	(10) Posterior pole rounded or sometimes pointed	8
8	(9) Nucleoli 8 in number. Nucleus pear-shaped (L $\times$ B) $32 \times 10 \mu$ . Body (L $\times$ B) $330 \times 68 \mu$	P. intestinalis. (Stein).
	(8) Number of nucleoli undetermined. Oval nucleus, $(L \times B) 23 \times 13 \mu$ Body $(L \times B) 288 \times 63 \mu$	P. orientalis Met.
10	(7) Posterior pole mamillionated. Nucleoli 20 in number. Nucleus pear-shaped, (L $\times$ B) 27 $\times$ 14 $\mu$ . Body (L $\times$ B) 275 $\times$ 57 $\mu$	P. indica sp. nov.
2	(1) Posterior pole tapering to a broadly rounded point	11
11	(12) Nuclei close together in centre. Nucleus oval $(L \times B)$ 18 $\times$ 10 $\mu$ . Body $(L \times B)$ 230 $\times$ 46 $\mu$ .	
	Nucleoli number not mentioned	P. yunnanensis Met.
12	(11) Nuclei far apart	13
13	(14) With definite number of nucleoli.—	
	(a) Nucleoli 6 in number. Body (L $\times$ B) 170 $\times$ 24 $\mu$ . Nucleus (L $\times$ B) 14 $\times$ 9 $\mu$	P. stejnegeri Met.
	(b) Nucleoli 8 in number. Slender Body (L $\times$ B) 97 $\times$ 18 $\mu$ . Nucleus ellipsoidal (L $\times$ B), 10 $\times$ 6 $\mu$	P. pelobatides Met.
	(c) Nucleoli 4 in number. Body (L $\times$ B) 240 $\times$ 68 $\mu$ . Nucleus (L $\times$ B) 25 $\times$ 8.9 $\mu$	P. dorsalis (Raff).
	(d) Nucleoli 3 in number. Nucleus (L $\times$ B) 6 $\times$ 2 $\mu$ , (anaphase). Body (L $\times$ B) 63 $\times$ 11 $\mu$	P. caccosterni Fantham.
	(13) With an undetermined number of nucleoli	15
15	(16) Nucleus ellipsoidal (L $\times$ B) 10 $\times$ 6 $\mu$ . Body (L $\times$ B) 160 $\times$ 32 $\mu$	P. peronii Met.
	(15) Nucleus rounded	17
17	(18) Nucleus smaller (diameter) 10 $\mu$ . Body (L $\times$ B) 572 $\times$ 70 $\mu$	P. hylarum (Raff).
18	(17) Nucleus large (diameter) 19 $\mu$ . Body (L $\times$ B) 313 $\times$ 70	P. luzonensis Met.

### Protoopalina karnatakensis, sp. nov.

Description.—The body is somewhat cylindrical with its anterior end elongated and bent to one side. One side of the body is slightly curved while the other is nearly straight. The posterior and the anterior poles are broadly rounded.

Two forms, namely, (i) slender and long, and (ii) stocky and broad were recognised. The cytoplasm is very well differentiated into the cortical and the medullary zones. The medulla is dense and contains endospherules which are small and sparse. The two nuclei almost spherical in shape, are placed quite apart along the vertical axis of the body. The posterior nucleus is situated somewhat away from the middle of the body, while the anterior nucleus is located more or less near the compact anterior region as in *P. indica*. The nuclear strand could not be traced at all. The chromatin of the nucleus consists of 4-6 nucleoli. The cilia are long and fine, but are shorter as compared to those of *P. indica*.

### Host.—Microhyla ornata.

Measurements in microns.—16 individuals. (slender and elongated): length  $\times$  breadth (average), 167  $\times$  47. 14 Zelleriella forms measured: length  $\times$  breadth (average), 85  $\times$  35. Diameter of the nucleus, 13.5. Endospherules (diameter) 1-2. Cilia line interval, 1.5-3.2.

Width of ectosarc 5.5.

Comparison.—Because of its flattened body form and dumbell-shaped nuclei, P. karnatakensis belongs to the sub-generic group V of Metcalf.

The species of this group are arranged in key below:—

1 (8)	Nuclei spindle-shaped or elliptical	2
2 (5)		
	• •	ů
3 (4)	Nuclei connected by a thread, elliptical (L $\times$ B) 13 $\times$ 3 $\mu$ . Body (L $\times$ B) 106 $\times$ 26 $\mu$	P. mossambicensis Met.
4 (3)	Nuclei not connected by a thread, Long nucleus (L $\times$ B) 17 $\times$ 6 $\mu$ . Body (L $\times$ B) 76 $\times$ 30 $\mu$	P. longinucleata Met.
5 (2)	Body broadened anteriorly and sharply pointed behind	6
6 (7)	Nucleoli 8 in number. Body (L $\times$ B) 196 $\times$ 30 $\mu$ . Nucleus (L $\times$ B), 30 $\times$ 13 $\mu$	P. regularis Met.
7 (6)	Nuclei 6 in number. Body (L $\times$ B) 119 $\times$ 30 $\mu$ . Nucleus (L $\times$ B) 22 $\times$ 8 $\mu$	P. rhinodermatos Met.
8 (1)	Nuclei ovoidal or dumbell-shaped	9
9 (12)	Posterior pole with a narrow posterior process	10
10 (11)	Nuclei 4 in number. Body (L $\times$ B) 90 $\times$ 40 $\mu.$ Nucleus (L $\times$ B) 27 $\times$ 6.2 $\mu$	P. xamachana Met.
11 (10)	Nucleoli 6-10 in number. Body (L $\times$ B) 102 $\times$ 42 $\mu$ . Dumbell-shaped nucleus, (L $\times$ B) 17 $\times$ 12 $\mu$	P. bufonis Met.
`´ı	Posterior pole broadly rounded. Nucleoli 4-6 in number. Slender forms $(L \times B)$ $167 \times 47$ $\mu$ . Zelleriella forms $(L \times B)$ $85 \times 35$ $\mu$ . Nucleus	
	ovoidal $13.5 \times 13 \mu$	P. karnatakensis, sp. nov.

### Protoopalina dharwarensis. sp. nov.

Description.—The body as compared to the previous species (P. indica and P. karnatakensis) is much elongated and more slender. The anterior and the posterior poles are broadly rounded and look alike. The granular cytoplasm is not well differentiated into ectosare and endosarc as in P. indica and P. karnatakensis. The spherules in the endosarc are fine and appear somewhat elliptical in form. Often, some minute chromatinlike bodies appear to group together towards the posterior end of the body. A group of vacuoles (constituting the excretory system) are also seen at this region. The two round nuclei are placed in the anterior half of the body. In some individuals, a halo is often present round the nuclei. The chromatin consists of small and irregular lits. Approximately 6 nucleoli were observed in one of the dividing nuclei. The nuclear thread was absent.

# Host.—Microhyla ornata.

Measurements in microns.—15 individuals. Body (L×B): average,  $207 \times 25$ ; L: B 8.02: 1. Nucleus (i)  $11.5 \times 9$ , (ii)  $13.5 \times 11$ , (iii)  $12.5 \times 9.5$ .

Diameter of endo-spherule, anterior region 1.5; posterior region 2.2. Cilia line interval, anterior region 1.5-2; posterior region, 3.3. Width of ectosarc 2.

Because of the bodyform, the species is referred to the subgeneric group IV, the key to the species of wich is given below.

# Key to the species of Group IV

- 1 (4) Nuclei pear-shaped 2 2 (3) Nucleoli apparently 6 in number. Body filiform
- (L  $\times$  B) 300  $\times$  35  $\mu$ . Nuclei connected by a thread, Nucleus (L  $\times$  B) 25  $\times$  6  $\mu$

3 (2) Nucleoli 10 in number. Body flattened (L  $\times$  B) 238  $\times$  22.8  $\mu.$  Nuclei (L  $\times$  B) 22.8  $\times$  9.5  $\mu$ 

4 (1) Nuclear shape otherwise

5 (6) Nuclei elliptical. Body very much attenuated. "Nucleoli in the form of 16 small chromatin masses." Body (L  $\times$  B) 530  $\times$  36  $\mu$ . Nucleus (L  $\times$  B) 24  $\times$  9  $\mu$  ...

6 (5) Nuclei oval or round. Body elongated and slender. Nucleoli apparently 6 in number. Nucleus reticulate (L  $\times$  B) 11·5  $\times$  9  $\mu$ . Body (L  $\times$  B) 207  $\times$  25

P. filiformis Met.

P. limnocharis, Nie.

P. tenuis (Raff).

P. dharwarensis, sp. nov.

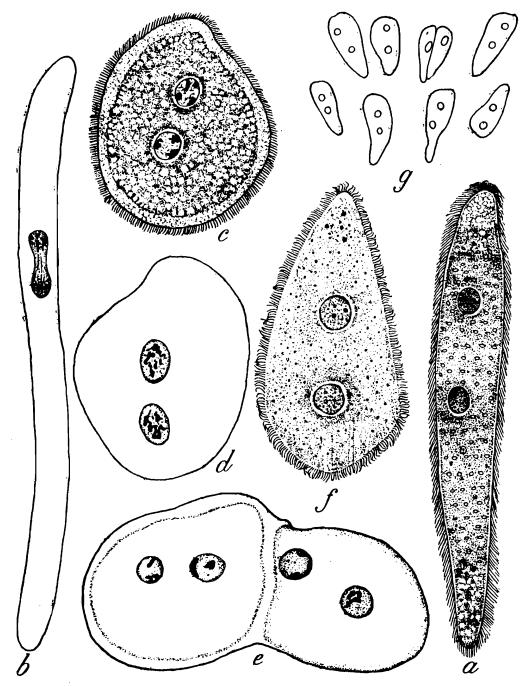
# Zelleriella microhylae, sp. nov.

Description.—The body is round and flat. The posterior projection as reported by Nie (1935) in Z. orientalis is not present. The coarsely alveolated cytoplasm is differentiated into a thin ectosarc and a dense endosarc. The small, oval and well stained endospherules give the endosarc a dense appearance. The two nuclei almost spherical are obliquely placed over one another in the cytoplasm. In the normal state, the nucleoli (4-6 in number) appear as large irregular bits of chromatin hanging from the surface of the nucleus. The cilia are short, fine and uniformly distributed all over the body. Various stages of mitotic divisions of the nucleus were observed

Host.—Microhyla ornata.

Meisurements in microns.—34 individuals.

Body (length  $\times$  breadth): range 70-110  $\times$ 60-80; average (L  $\times$  B)  $\times$  70, Length: Breadth (average) 1.28:1; Dividing individual (L  $\times$ B),



Text-fig. 2. a and b.—Protoopalina dharwarensis sp. nov. a. Normal individual showing general structure:  $\times$  430; b. Daughter cell from a longitudinal division showing dumbell-shaped nucleus:  $\times$  430. c-d.—Zelleriella microhylae, sp. nov. c. Normal individual showing general structure:  $\times$  430; d. Nuclei in prophase:  $\times$  430; e. Dividing individuals with nuclei in telophase:  $\times$  430. f. & g.—Zelleriella froilanoi, sp. nov. f. Normal individual showing general structure:  $\times$  430; g. Dividing individuals under low power:  $\times$  100.

150 × 90. Diameter of the resting nucleus, 10-13. Cilia line interval: anterior region, 3; posterior region, 4. Length of the cilia, 8. Width f ectosarc, 4-4.5.

# Zelleriella froilanoi, sp. nov.

Description.—The body is thin, flat and bluntly rounded at the pos-The anterior pole is broad and round. The posterior one is tapering to a rounded point. The small and stocky forms have somewhat a wedge-shaped body. Unlike Z. microhylae, the cytoplasm is not well differentiated into the usual zones. The protoplasm is very finely granulated and is evenly distributed. Cytoplasmic inclusions in the form of very fine granules which stain like chromatin were seen in some individuals. The oval endospherules are small and sparsely distributed in the cytoplasm. Some small and large vacuoles are often seen to group together at the posterior pole of the body. spherical nuclei are placed one over the other in the vertical axis of the The chromatin is found to be in the form of minute granules and not in large bits as seen in Z. microhylae. In the dividing nucleus the nucleoli were approximately 8 in number but usually varied from 6-8.

Host.—Philautus sp.

Measurements in microns.—71 individuals.

Body (length  $\times$  breadth): range 80-150  $\times$  25-55; average (L  $\times$  B), 115  $\times$  40: Length: Breadth (average) 2.87:1; diameter of the nucleus 10-12. Distance between the two nuclei 15-30. Length of the cilia, 12. Cilia line interval, 3.

# Key to the species of Zelleriella.

1 (13) Body without a posterior curved process	2
2 (7) Body with a posterior end narrowly elongated and pointed.—	
(a) Body, trumpet-shaped, posterior end elongated. Body (L $\times$ B), 180—220 $\times$ 75—100 $\mu$ . Nucleus spherical, 20-22 $\mu$ diameter	Z. cornucopia Carini.
(b) Body cone-shaped. Posterior pole rounded. Nucleus spherical 15-17 $\mu$ in diameter; distance between two nuclei 20-25 $\mu$ . Body (L $\times$ B), $100 \times 30 \mu$	Z. falcata Carini.
(c) Body comma-shaped. Posterior pole pulled to a point. Body 70-90 μ in length. Diameter of the spherical nuclei, 8-10 μ; distance between two nuclei, 10 μ	Z. corniola Carini.
(d) Body wedge-shaped	3
3 (4) Nuclei ellipsoidal or pear-shaped (number of nucleoli not mentioned); Body (L $\times$ B) $207 \times 130 \mu$ . Nucleus (L $\times$ B) $32 \times 22 \mu$	Z. magna Met.
4 (3) Nuclei spherical	5
5 (6) Body very thin and broadly wedge-shaped; L $\times$ B, 115 $\times$ 40 $\mu$ . Nucleus diameter 10-12 $\mu$ . Nucleoli 6-8 in number. Distance between two nuclei, 15-30	
μ	Z. froilanoi, sp. nov.
6 (5) Body narrowly wedge-shaped. Body (L $\times$ B), 93 $\times$ 50 $\mu$ . Nucleoli 4 in number. Nucleus diameter 10 $\mu$	Z. intermedia Met.
7 (2) Body with a posterior end broadly rounded.—	2. thier meata Met.
(a) Body irregularly rounded, thin and leaf-like. L $\times$ B, 300 $\times$ 200 $\mu$ . Nucleoli 8 in number. Spherical nucleus 25 $\mu$	7. f.1:
Sphortean nuclous 20 k	Z. foliacea Carini.

		Z. truncata Carini.
	(c) Body oval and greatly flattened	8
8 (12	2) Nuclei rounded—	
	(a) Nuclei large, 20 $\mu$ in diameter	Z. binucleata (Raff.
9 (8	B) Nuclei smaller	10
10 (1)	l) Nucleus 12 μ. in diameter. Nucleoli 3-4 number	Z. macronucleals Bezz.
11 (10	Nucleus 10-13 $\mu$ in diameter. Body thick (L $\times$ B), 70-110 $\times$ 60-80 $\mu$ . Nucleoli 4-6 in number	Z. microhylae, sp. nov.
12 (8	Nuclei ovoidal (L $\times$ B) 15 $\times$ 10 Nucleoli 4 in number. Body (L $\times$ B) 130 $\times$ 82 $\mu$	Z. brasiliensis (Pinto).
13 (	l) Body with a posterior curved process	14
14 (18	75) Posterior tail long and distinct, Nuclei (L $\times$ B) 23 $\times$ 13.9 $\mu$ . Body (L $\times$ B) 100 $\times$ 60 $\mu$	Z. antanesi Pessoa.
15 (14	Posterior tail otherwise	16
16 (17	7) Posterior end terminates to a minute sharp point. Nucleoli 8 (?) in number. Body leaflike, (L $\times$ B) 87.5.120 $\times$ 45.70 $\mu$ . Nucleus 12.5 $\mu$ in diameter	Z. orientalis Nie.
17 (1	6) Body broad anteriorly. Nucleoli 3 in number. Nucleus 10 $\mu$ in diameter. Body (L $\times$ B) 105 $\times$ 80 $\mu$	Z. bufonix Met.

#### SUMMARY.

- 1. The ciliate parasites of about one hundred and twenty-five specimens of frogs and toads of Karnatak, Bombay Presidency, comprising nine different species have been studied particularly with a view to ascertain the presence of binucleated opalinids in them.
- 2. Five new species of Zelleriella and Protoopalina have been described, thus establishing, beyond doubt, the presence of binucleated opalinids in these anurans.
- 3. Ciliate parasites of six of the host species have been recorded for the first time in India; and some more ciliates have been added to the list of parasites of the hosts already examined. Besides one new species and one new sub-species of the genus Cepedea, ten old species of ciliates found parasitic in the gut of these anura have also been recorded.

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