

**The Life Cycle and Morphology of *Tetracerasta blepta*,  
gen. et. sp. nov., and *Stegodexamene callista*, sp. nov.  
(Trematoda : Lepocreadiidae) from the Long-Finned  
Eel, *Anguilla reinhardtii* Steindachner**

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*Abstract*

Two new lepecreadiid digeneans, *Tetracerasta blepta*, gen. sp. nov., and *Stegodexamene callista*, sp. nov., are described from the intestine of the long-finned freshwater eel, *Anguilla reinhardtii*, in the Brisbane River, Queensland, and from the Australian bass, *Macquaria novemaculeata*, in the Richmond River in New South Wales. Their life cycles have been elucidated and completed in the laboratory, by means of uninfected hosts. Both lepecreadiid species use the prosobranch gastropod, *Posticobia brazieri*, as their first intermediate host. Cercariae of *T. blepta* penetrate and encyst in the pharyngeal muscle, external muscle, and viscera of fishes in the genera *Gobiomorphus* and *Hypseleotris*, and in several species of tree frog tadpoles. The cercariae of *S. callista* are often eaten or accidentally inhaled by several small fishes, including *Retropinna*, *Craterocephalus*, *Pseudomugil* and *Ambassis*, and encyst in the pharyngeal muscle and viscera. All developmental stages are described and illustrated.

**Introduction**

Lepocreadiidae Nicoll, 1934, are digenean parasites of marine fishes, characterized by their spiny tegument and the presence in their life cycle of rediae which produce hairy-tailed cercariae without stylets. Although Yamaguti (1975) provided a good summary of most of the, approximately 20, known life cycles, important recent contributions to knowledge of these cycles are from Stunkard (1980a, 1980b) and Bartoli and Prévot (1978).

Macfarlane (1951, 1952) described the morphology, life cycle and bionomics of the lepecreadiid *Stegodexamene anguillae* from two species of New Zealand freshwater eels, *Anguilla dieffenbachii* Grey and *A. australis schmidtii* Phillipps.

The purpose of this study was to elucidate the life cycles and to describe the morphology of two lepecreadiid species found initially by Pearson (personal communication) in the long-finned freshwater eel, *Anguilla reinhardtii* Steindachner, in the Brisbane River system. As Pearson believed, one trematode was found to be a new genus and the other a new species of the existing genus *Stegodexamene* Macfarlane, 1951.

**Materials and Methods**

A record of the wild hosts examined for parasites, and of the parasite-free hosts used in experimental infections, appears in Table 1. The numbers of parasites recovered from experimental infections were, necessarily, estimates in some cases, such as when experimentally infected fishes were used whole to infect eels so as to minimize the loss of metacercariae.

Infection-free hosts were used throughout the life-cycle studies. The snails, *Posticobia brazieri*, were laboratory-reared. Rainbowfish, *Nematocentris fluvialis*, which were raised in pools without *P. brazieri*, were used as the intermediate hosts of one lepecreadiid, and young unparasitized tadpoles of *Litoria lesueuri* were used for the other.

As each stage of the life cycles of *T. blepta* and *S. callista* was completed in the laboratory, hosts were examined for parasites, and some parasites would be used for observation or measurement while others

Table 1. Numbers of wild and experimental hosts infected with the parasites *Tetracerastra blepta* (T) and *Stegodexamene callista* (S)

Host species	No. of wild hosts Examined	No. of wild hosts Infected	Life cycle stage	Hosts used	Numbers in laboratory infections Hosts infected	Parasites given	Parasites recovered
<i>Posticocöta brazieri</i>	8100 <sup>A</sup>	T, 500 <sup>A</sup> S, 500 <sup>A</sup>	Sporocyst Redia	700 <sup>A</sup> 500 <sup>A</sup>	280 <sup>A</sup> 300 <sup>A</sup>	? ?	? ?
<i>Thiara</i> sp.	50 <sup>A</sup>	0	—	—	—	—	—
<i>Physastra</i> sp.	20 <sup>A</sup>	0	—	—	—	—	—
<i>Physa</i> sp.	20 <sup>A</sup>	0	—	—	—	—	—
<i>Ambassis</i> sp.	15	T, 0 S, 1	Metacercaria	1	0	?	0
<i>Bufo marinus</i> (introduced toad)	30	T, 0 S, 0	Metacercaria	1 5	1 0	? 25 <sup>A</sup>	2 0
<i>Cratogeomys merriami</i>	13	T, 0 S, 3	Metacercaria	1 1	0 0	30 <sup>A</sup> 111	0 0
<i>Gambusia affinis</i> (introduced fish)	21	0	—	—	—	30	0
<i>Gobiomorphus australis</i>	5	T, 5 S, 0	Metacercaria	—	—	—	—
<i>Gobiomorphus</i> sp.	12	T, 0 S, 0	Metacercaria	1	1	127	14
<i>Hypseleotris compressus</i>	25	T, 1 S, 0	Metacercaria	1	0	146	0
<i>H. galli</i>	15	T, 6 S, 0	Metacercaria	4 2	2 2	200 30	33 5
<i>Litoria</i> spp. tadpoles < 3 weeks old	45	T, 0 S, 0	Metacercaria	150 <sup>A</sup> 90 <sup>A</sup>	135 <sup>A</sup> 80 <sup>A</sup>	? —	2000 <sup>A</sup> —
> 3 weeks old	20	T, 3 S, 0	—	—	—	—	—
<i>Mixophyes</i> sp. tadpole	2	T, 1 S, 0	Metacercaria	—	—	—	—

<i>Mugil cephalus</i>	10	0	—	—	—	7 <sup>A</sup>	700 <sup>A</sup>	—	13 <sup>A</sup>
<i>Nematocentris</i> spp. (pond-reared)	38	T, 0	Metacercaria	9	—	9 <sup>A</sup>	1300 <sup>A</sup>	—	37 <sup>A</sup>
<i>Pseudomugil signifer</i>	25	S, 0	Metacercaria	16	—	—	—	—	—
<i>Retropinna semoni</i>	22	T, 0	Metacercaria	1	—	1	113	—	4
		S, 3				0	100 <sup>A</sup>	—	0
		T, 0		1	—	1	300 <sup>A</sup>	—	20
		S, 0		3	—	—	—	—	—
<i>Tandanus tandanus</i>	16	0	—	—	—	—	—	—	—
<i>Anguilla australis</i>									
From Brisbane	4	0	—	—	—	—	—	—	—
From Victoria	26	T, 0	Adult	8	—	7	650 <sup>A</sup>	—	59
		S, 0		8	—	4	750 <sup>A</sup>	—	36
<i>A. reinhardtii</i> adults									
From Brisbane River	30	T, 19	Adult	—	—	—	—	—	—
		S, 29							
From University of Queensland	21	T, 0	Adult	7	—	3	400 <sup>A</sup>	—	11
		S, 0		2	—	2	50 <sup>A</sup>	—	7
From Victoria	21	T, 0	—	—	—	—	—	—	—
		S, 12							
<i>A. reinhardtii</i> elvers, Queensland	3	T, 0	—	—	—	—	—	—	—
		S, 1	Adult	—	—	—	—	—	—
<i>A. reinhardtii</i> glass eel, Queensland	80	T, 0	Adult	2	—	2	?	—	4
		S, 0		2	—	1	?	—	3
<i>Leiopotherapon unicolor</i>	18	T, 0	—	—	—	—	—	—	—
		S, 0	Adult	2	—	0	6	—	0
<i>Macquaria novemaculeata</i> , N.S.W.	19	T, 9	Adult	—	—	—	—	—	—
		S, 12		—	—	—	—	—	—
<i>Glossamia aprion</i>	10	T, 0	Adult	1	—	0	2	—	0
		S, 0		—	—	—	—	—	—

<sup>a</sup> Estimated number only.

would be used to infect the next host in the life cycle. In this way, eggs of these trematodes recovered from adults in the intestines of wild *A. reinhardtii* were used to initiate infections which developed in parasite-free intermediate hosts and resulted in gravid adults in previously parasite-free *A. australis*. To ensure that these eggs were healthy, they were allowed to develop and the life cycle repeated until metacercariae developed for the second time.

All hosts taken from the wild were not assumed infection-free until at least 20 of a range of sizes were dissected and found free of all lepecreadiid or other digeneans that might be confused with them. Such was the case for the eels *Anguilla australis* taken from the Merri Creek, Yarra River, near Melbourne in Victoria, and *A. reinhardtii* from the University of Queensland's lake, Brisbane, which were both used as definitive hosts in the life-cycle studies.

Eels used for study of the disposition of adult lepecreadiids along the gut were dissected within 12 h of capture and were alive until just before dissection.

More than 8000 *P. brazieri* were observed individually or in small groups for cercarial emergence. Normally these were examined once a day in the morning, but to establish the period of emergence snails were examined hourly during the day and at approximately 6-h intervals during the evening.

Most observations were made on living specimens, unless otherwise stated. All morphological measurements were made on specimens killed and fixed in 5–10% formalin, stained in haemalum, dehydrated with ethanol, cleared in methyl benzoate and mounted in canada balsam. Specimens were not flattened.

Unless otherwise stated, all measurements are reported in micrometres in the form: average (range, sample size).

The development of miracidia was followed by placing eggs in a small volume of water under a coverslip supported and sealed by petroleum jelly. The epidermal plates and pores of miracidia were demonstrated by the method of Lynch (1933). Miracidia were examined head-on by a modification of Anderson's (1958) method.

In preparation for scanning electron microscopy, specimens were critical-point dried while enclosed in fine netting to prevent loss, then sputter-coated.

The key to the abbreviations of museums is as follows: BMNH, British Museum (Natural History), London; MPMT, Meguro Parasitological Museum, Tokyo; QM, Queensland Museum, Brisbane; SAM, South Australian Museum, Adelaide; USNM, United States National Museum, Beltsville, Maryland.

Abbreviations in the Figures are as follows:

a.	apical gland	l.c.	Laurer's canal
a.b.	anterior end of bladder	m.	muscle
a.g.	accessory gland	m.g.	metraterm gland
a.s.	anterior seminal vesicle	o.	ovary
b.	excretory bladder	oe.	oesophagus
b.p.	birth pore	o.l.	oral sucker lobe
c.	caecum	op.	operculum
c.e.	cercarial embryo	o.s.	oral sucker
c.g.	cystogenous gland	p.	pharynx
ci.	cirrus	pa.	papilla(e)
cl.	cilia	p.c.	pigment cup
cr.	crystals	p.g.	penetration gland
c.s.	cirrus sac	p.g.d.	penetration gland duct
c.w.	cyst wall	p.g.p.	penetration gland pore
e.	egg(s) in uterus	p.l.	protracted sucker lobe
e.p.	excretory pore	po.	pores
ex.s.	excretory sphincter	p.p.	pars prostatica
ey.	eyespot	p.s.	posterior seminal vesicle
f.c.	flame cell	r.e.	radial embryo
f.d.	flagella in main duct	r.f.c.	radial flame cell
g.c.	gland cell(s)	rh.	rhabdomere
g.d.	gland ducts	r.l.	retracted sucker lobe
g.o.	genital opening	r.p.	rings of papillae
h.c.	host capsule	s.	sporocyst
l.	lappet		

s.c.	sucker of cercaria	u.	uterus
s.d.	sperm duct(s)	v.	vitelline follicle(s)
s.f.c.	sporocyst flame cell	v.c.	vitelline cell
s.g.	skin gland	v.m.	vitelline membrane
s.r.	seminal receptacle	v.r.	vitelline cell remnants
s.v.	seminal vesicle	v.s.	ventral sucker
t.	testes	y.r.	yolk reservoir
ta.	tail	z.	zygote

### Genus *Tetracerasta*, gen. nov.

Type- and only species: *Tetracerasta blepta*, sp. nov.

Origin of name: Greek, plural, neuter, meaning four horns, describing the four oral sucker lobes.

### Diagnosis

With characteristics of family Lepocreadiidae (Odhner, 1905) Nicoll, 1934; cylindrical, scale- and spine-covered. Oral sucker larger than ventral sucker, with dorsal transverse row of 4 extensible lobes with conical ampullae connected to extensive gland cells in anterior body. Prepharynx very short; oesophagus long; caeca long, end blindly at posterior end. Testes 2, oblique to nearly tandem; cirrus sac large, continues posterior to ventral sucker; seminal vesicle bipartite, no external seminal vesicle; pars prostatica well developed; cirrus spiny; uterus pre-ovarian; petal-like metraterm glands form semicircle around end of uterus; genital opening anterosinistral to ventral sucker; eggs large and few; vitellaria confined posterior to base of cirrus sac. Bladder tubular, extending from bifurcation of caeca to opening at posterior end. Cercaria trichocercous.

### Comments

*Tetracerasta* is close to *Stegodexamene*, but differs from it in having oral sucker lobes with associated glandular tissue and a larger oral than ventral sucker. Absence of an external seminal vesicle differentiates *Tetracerasta* from *Lepocreadium* and *Opechona*.

### *Tetracerasta blepta*, sp. nov.

(Figs 1, 3, 5, 7)

### Types

Holotype, QM (GL 1561). Paratypes: 2, QM (GL 1562-3); 3, SAM (V3078-80); 3, BMNH (1982.3.11.4-6); 3, USNM (77029); 3, MPMT (MPM 19355). All type specimens are whole mounts.

*Host.* *Anguilla reinhardtii*.

*Site in host.* Small intestine.

*Geographical distribution.* Brisbane and Fitzroy Rivers, Qld. Richmond River, N.S.W.

*Other hosts.* *Macquaria novemaculeata*; *Anguilla australis* (experimental infections).

### Description

Based on observation of live material, supplemented by measurements of 15 fixed specimens (type series) from hindgut of *Anguilla reinhardtii*.

Adult with taxonomic characters of family; cylindrical (Fig. 1); length 2010 (1715-2412, 12), widest at ventral sucker 228 (195-251, 12); anterior surface covered by scales (Fig. 7)



which become spines posteriorly, extending to level of ovary and sparsely to posterior end. Oral sucker large, subterminal, ventral, retractile, oval; length 150 (128–182, 15), width 173 (150–205, 12), with glandular tissue on dorsal edge; 4 lobes in transverse row on dorsal margin. Lobes extensible (Fig. 7), with conical ampullae apically (Fig. 50). Ventral sucker smaller, circular, 110 (82–146, 12), with 3 pointed papillae forming triangle in addition to less constant papillae (Fig. 1). Skin glands sparse, only anterior to ventral sucker.

Prepharynx very short (Fig. 1); pharynx ovoidal, length 53 (46–77, 15), width 67 (58–84, 12); oesophagus length 206 (144–397, 15), width 17 (8–31, 12), bifurcation anterior to ventral sucker; caeca end blindly at posterior end, length 1479 (1348–1776, 15), width 20 (10–23, 12).

Testes 2, ovoidal, oblique to nearly tandem, posterior to ovary; anterior testis sinistral, length 118 (90–157, 15), width 92 (67–113, 12); posterior testis dextral, length 127 (102–157, 15), width 98 (77–123, 12); sperm ducts separate, join at posterior end of cirrus sac; cirrus sac botuliform (Fig. 3), extends from halfway between ovary and ventral sucker dextrad to just anterior to ventral sucker, length 242 (195–335, 15), width 75 (63–104, 11). Cirrus sac muscular enclosing bipartite seminal vesicle; posterior seminal vesicle oval, length 137 (102–197, 15), width 63 (51–84, 15), sperm-filled; anterior seminal vesicle triangular to oval, length 74 (38–102, 15), width 43 (26–64, 15), sperm-filled; pars prostatica triangular, length 74 (59–102, 11), width 36 (26–44, 15); cirrus length variable, width 43 (36–59, 12), 12 spinous patches in folds distally, spines in lumen just anterior to pars prostatica, probably evaginates to pars prostatica; genital sinus small; genital opening 27, anterosinistral to ventral sucker; metatrerm glands unicellular, 6–10, petal-like, in semicircle surrounding end of the uterus on anterior, sinistral and posterior sides, immediately dorsal to genital opening; accessory gland cells surrounding metatrerm glands anteriorly, externally and posteriorly.

Ovary oval, length 76 (65–92, 15), width 70 (42–92, 12), submedian, dextral, posterior to ventral sucker (Figs 1, 5); seminal receptacle lacrimiform, sperm-filled, length 89 (63–136, 15) width 44 (27–54, 12); Laurer's canal 78 long, 8 wide, narrow, dextral, opposite seminal receptacle (Fig. 5), opening sinistral; yolk reservoir oval, length 41 (21–56, 14), width 54 (42–73, 12), sinistral to ovary vitelline follicles surround caeca dorsally, ventrally and externally, extend from level of anterior testis to end of caeca, confluent posterior to testes; follicles oval, length 45 (33–54, 15), width 27 (17–33, 12); uterus entirely anterior to ovary, terminal metatrerm, 3–5 loops, contains 16 (50–45, 36) eggs; eggs smooth, light brown, ovoid, length 70 (62–77, 16), width 48 (47–52, 16).

Flame cell formula  $2[(3+3+3)(3+3+3)]$ ; anterior and posterior collecting ducts join main ducts anterolateral to ventral sucker, at level of bifurcation of caeca; main ducts join excretory bladder anterior to ovary. Bladder long, tubular, extends from bifurcation of the caeca, dorsal to the ovary and testes, to posterior end; length 1458 (1321–1674, 15), width 63 (27–94, 12), excretory pore terminal.

Eyespot pigment widely scattered in forebody.

### Development

The least mature adults examined, 7 days after infection, were less mature than the most developed metacercariae. Experimental infections of *T. blepti* developed normally in the eel *A. australis* for 1 month or less, at 20–25°C, then stopped growing and died before they became gravid. In *A. reinhardtii*, worms became gravid 19–47 days after infection depending on the age of the metacercariae used; older metacercariae developed into mature adults sooner. Infections did not survive longer than 2 months in the laboratory.

### Disposition in the Gut of the Eel

Although *T. blepti* was found along the entire length of the intestine and rarely in the stomach of *A. reinhardtii*, distribution was not uniform (Table 2). Two-thirds were found in the first quarter of the intestine and most of the remaining worms in the second quarter.

*T. blepti* is similarly distributed in the Australian bass, *M. novemaculeata*.

Genus *Stegodexamene* Macfarlane*Stegodexamene callista*, sp. nov.

(Figs 2, 4, 6, 8, 9)

*Types*

Holotype, QM (GL 1564). Paratypes: 2, QM (GL 1565-6); 3, SAM (V3081-3); 3, BMNH (1982.3.11.1-3); 3, USNM (77029); 3, MPMT (MPM 19356). All type specimens are whole mounts.

*Host.* *Anguilla reinhardtii*.

*Site in host.* Small intestine.

*Geographical distribution.* Brisbane River, Qld; Richmond River, N.S.W.; Franklin and Agnes Rivers, Vic.

*Description*

Based on observation of live material supplemented by measurements of 16 fixed specimens (type series), from the hindgut of *Anguilla reinhardtii*.

Adults with characteristics of family and genus. Body flattened (Fig. 2), length 1720 (1450-2140, 16), width 227 (190-290, 16), anterior body surface covered with scales which become spines posteriorly and extend to level of ovary and sparsely to posterior end. Oral sucker subterminal, ventral, retractible, oval (Fig. 8); length 73 (65-94, 16), width 80 (71-96, 16). Ventral sucker subequal, circular 82 (73-104, 16), with 2 concentric rings of papillae, inner with 5 small pointed, outer with 9 large blunt papillae and other scattered papillae of inconsistent distribution (Fig. 9). Skin glands numerous anterior to ventral sucker (Fig. 2).

Prepharynx very short; pharynx ovoidal, length 48 (38-56, 16), width 53 (46-63, 16); oesophagus length 183 (121-242, 16), width 10 (6-12, 16), bifurcation anterior to ventral sucker; caeca end blindly at posterior end, length 1408 (1135-1776, 16), width 29 (21-46, 16).

Testes 2, ovoidal, oblique, posterior to ovary; anterior testis sinistral, length 115 (102-136, 16), width 90 (73-104, 16); posterior testis dextral, length 124 (109-146, 16), width 96 (84-113, 16); sperm ducts separate, open separately and posterodorsally into cirrus sac; cirrus sac botuliform (Fig. 4), extends from halfway between ovary and ventral sucker dextrally to just anterior to ventral sucker, length 256 (214-316, 16), width 75 (63-104). Cirrus sac muscular, enclosing bipartite seminal vesicle; posterior seminal vesicle oval, length 124 (90-165, 16), width 65 (52-92, 16), sperm-filled; anterior seminal vesicle oval, length 51 (38-63, 16), width 44 (33-61, 16), sperm-filled; pars prostatica triangular, length 115 (86-146, 16), width 42 (36-52, 15); cirrus length variable, width 43 (36-52, 15), end of cirrus with small patches of spines, small spines in lumen, probably evaginates to level of pars prostatica; genital sinus small; genital opening 28, anterosinistral to ventral sucker; metraterm glands 6-10, large, unicellular, petal-like, surrounding terminal end of uterus sinistrally just dorsal to genital opening; accessory glands surround metraterm glands anteriorly, posteriorly and externally.

Figs 3, 4. Terminal genitalia of holotype adult of: 3, *T. blepta*; 4, *S. callista*.

Figs 5, 6. Female reproductive system of adult (composite drawn from live specimens): 5, *T. blepta*; 6, *S. callista*.

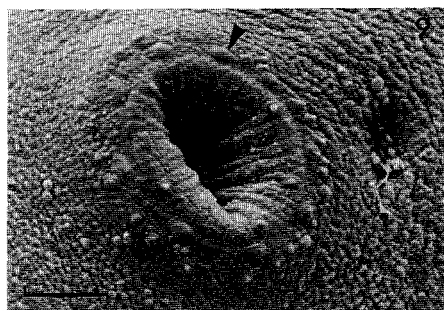
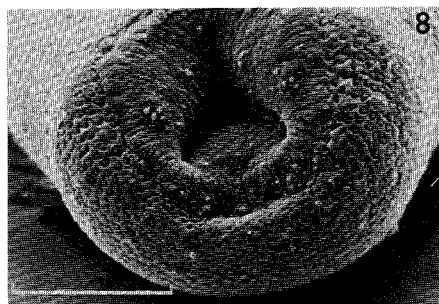
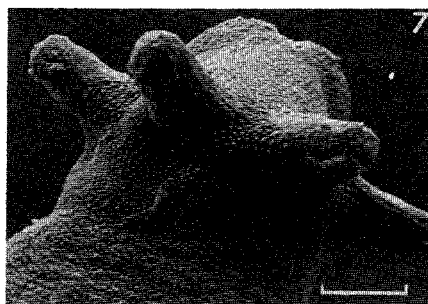
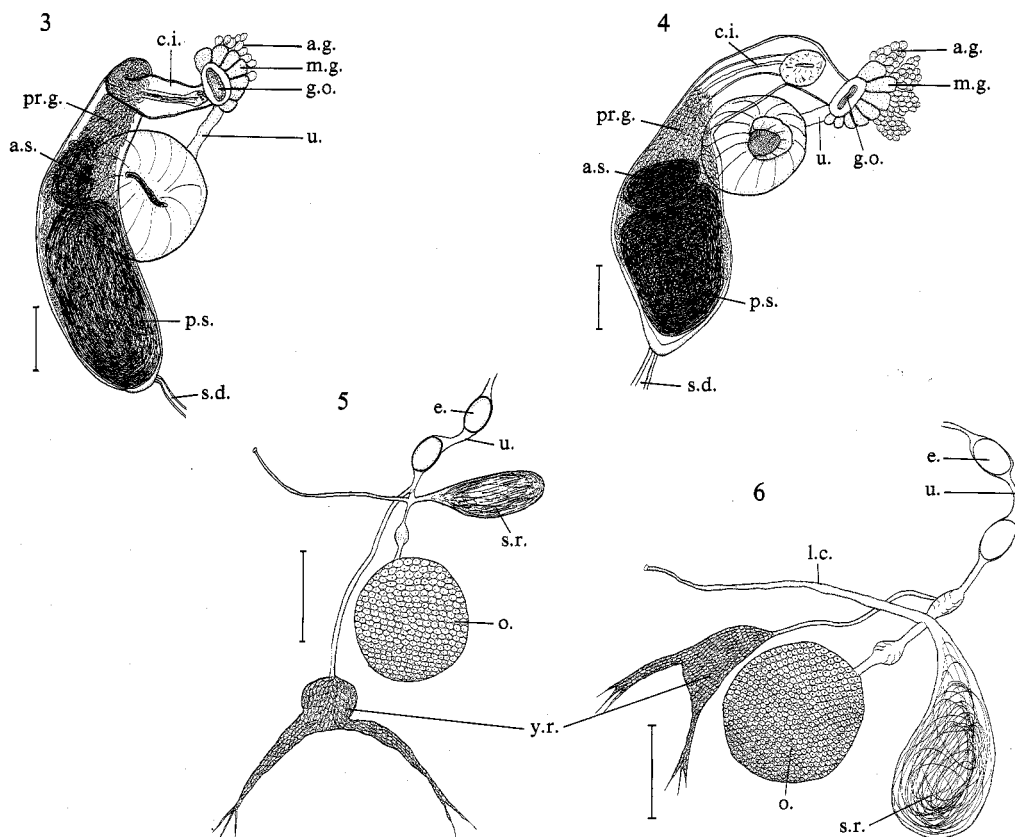
For abbreviations, see p. 180. Scale line, 50  $\mu$ m.

Fig. 7. *T. blepta*, anterior end of adult, with oral lobes.

Figs 8, 9. *S. callista*, adult: 8, oral sucker; 9, ventral sucker and genital opening; arrow, one of the papillae.

Scale lines: 7, 8, 40  $\mu$ m; 9, 20  $\mu$ m.





Ovary circular 77 (65–100, 16), submedian, dextral, posterior to ventral sucker, close to anterior testis; seminal receptacle lacrimiform, sperm-filled, length 85 (52–107, 15), width 50 (36–69, 15); Laurer's canal c. 80 long, narrow, connects to seminal receptacle (Fig. 6), opening sinistral, anterior to ovary; yolk reservoir oval, length 45 (21–75, 16), width 36 (17–50, 16), connects to oviduct; vitelline follicles surround caeca dorsally, ventrally and externally, extend from ovary to end of caeca, become confluent posterior to testes, ending just anterior to posterior end of body; follicles oval, length 30 (21–40, 16), width 23 (19–29, 16); uterus entirely anterior to ovary, 2–3 loops, contains 9 (4–29, 32) eggs; eggs smooth, light brown, ovoid, length 70 (64–74, 14), width (42–49, 14).

Flame-cell formula  $2[(4+5+5)(5+5+5)]$ ; anterior and posterior ducts join main ducts anterolateral to ventral sucker, anterior to bifurcation of caeca; main ducts join excretory bladder just anterior to ovary. Bladder long, tubular, extends from bifurcation of caeca or even just posterior to pharynx, dorsal to ovary and testes, to posterior end; length 1411 (1144–1776, 16), width 46 (29–75, 16).

Eyespot pigment widely scattered in forebody.

### Comments

The adults of *S. callista* and *S. anguillae* are very similar and share the characteristics of the genus as given by Macfarlane (1951) and as seen in specimens obtained from *Anguilla australis* from Christchurch, New Zealand. *S. callista* is a smaller worm, and its pars prostatica is not nearly as extensive as that of *S. anguillae*. The excretory bladder of *S. callista* may extend even anterior to the bifurcation of the caeca, whereas this is not reported for *S. anguillae*. The flame-cell pattern of *S. anguillae* illustrated by Macfarlane (1951) is  $2[(3+3)(3+3+3+3+3)]$  or 42 in total, although Macfarlane believed it might be incomplete. The flame-cell pattern for *S. callista* is  $2[(4+5+5)(5+5+5)]$  or 50 in total. As previously discussed, Macfarlane (1951) did not report cirrus spines for *S. anguillae*, as have been observed in *S. callista*, but such spines are easily overlooked. The cercaria of *S. callista* and *S. anguillae* differ as discussed below.

Yamaguti (1971) wrongly places *Rhynchocreadium*, a synonym for *Allocreadium*, as a subgenus of *Stegodexamene*. This is wrong because *Rhynchocreadium* lacks body spines and has only a simple seminal vesicle, a characteristic of the family Lepocreadiidae. Until the life history of *Rhynchocreadium* is known, I believe that the two species of this genus are identical and should be known as *Allocreadium mehra* Gupta, 1958, an allocreadiid, following the suggestions of Kakaji (1969).

### Development

Adults immediately after excystment were identical to well developed metacercariae. Egg production started at about 70 days after the metacercariae were ingested by the eel, at 20–25°C. A single adult remaining in a 192-day infection was thin and not active; the testes and vitelline follicles were reduced and there were very few eggs in the uterus, indicating that the worm was senescent. As only a single worm remained of the infection it is likely that older worms may cease to produce eggs and die.

When very young (less than 8-day-old) metacercariae were used to infect eels, the size and maturity of the adults at 60 days were comparable with those of adults developing from 4-week-old metacercariae, suggesting that development from young metacercariae is not a disadvantage and that compensatory growth occurs.

### Disposition in the Gut of the Eel

*S. callista* was more uniformly distributed along the intestine of the eel *A. reinhardtii* than was *T. blepta*, and was rarely found in the stomach (Table 2). Almost two-thirds of *S. callista*

were found in the second and third quarters of the intestine, with over one-third in the second quarter alone. Distribution along the intestine of the Australian bass, *M. novemaculeata*, is similar.

Typically, *A. reinhardtii* has three intestinal trematodes: *S. callista*, *T. blepta* and an unidentified opascoelid. The stomachs of larger eels often have unidentified hemiurids. In a typical infection the hemiurids are found exclusively in the stomach and pharynx along with large spirurid nematodes, probably *Proleptus*. In the first quarter of the intestine there are many *T. blepta*, and *S. callista* is about half as numerous. In the second quarter the numbers of *S. callista* peak, and there may be a few opascoelids. In the third and fourth quarters there are very few *T. blepta*. In the third quarter *S. callista* is numerous, and there may be a few opascoelids; in the last quarter there are few *S. callista* but many opascoelids.

If only one species, either *S. callista* or *T. blepta*, occurs within an eel, a rare occurrence except in experimental infections, then the worms are evenly distributed along the intestine, which suggests that species interaction restricts the distribution of the worms. *T. blepta* occur in the stomach only in an eel without the hemiurids that normally occupy that region. An eel without opascoelids has more leprocreadiids in the last quarter, where the opascoelids normally are most numerous.

**Table 2.** Distribution of adult digeneans along the gut of six freshly killed eels, *Anguilla reinhardtii*

Species	No. in stomach	No. in intestinal quarter			
		1	2	3	4
Unidentified hemiurid	27	0	0	0	0
<i>Tetracerasta blepta</i>	54	314	101	5	1
<i>Stegodexamene callista</i>	4	126	240	210	57
Unidentified opascoelid	0	0	2	6	253

Macfarlane (1951), Hine (1980) and Rid (1973) found that New Zealand freshwater eels, *Anguilla dieffenbachii* and *A. australis schmidtii*, normally have intestinal infections of two trematodes. *Stegodexamene anguillae* is found in the anterior one-third of the intestine and *Telogaster opisthorchis* in the posterior two-thirds (Macfarlane 1945, 1951). Hine (1980) found that if the eel had a heavy infection of *S. anguillae*, then the midgut was more heavily infected. In the present study, if *T. blepta* was numerous then its distribution was wider and continued into the latter half of the eel's intestine. An increase in the population and hence in the range of one leprocreadiid restricted the range of the other.

Macfarlane (1952) believed that differences in chemotropism, effective after *S. anguillae* and *T. opisthorchis* excyst, produced the site segregation. Hine (1980) believed that the species interacted, and he observed an extension of the *S. anguillae* population posteriorly due to crowding, which resulted in a limited anterior distribution of *T. opisthorchis*. This agrees with the present study.

Hine (1980) believed that the segregated distribution of *S. anguillae* and *T. opisthorchis* resulted from a posterior migration of *T. opisthorchis* after excystment, because it is unlikely that this species, with its thinner cyst wall, excysts more posteriorly than *S. anguillae*. Differences in the thickness of the host capsule or cyst wall are not believed to contribute to the segregated distribution of *S. callista* and *T. blepta*.

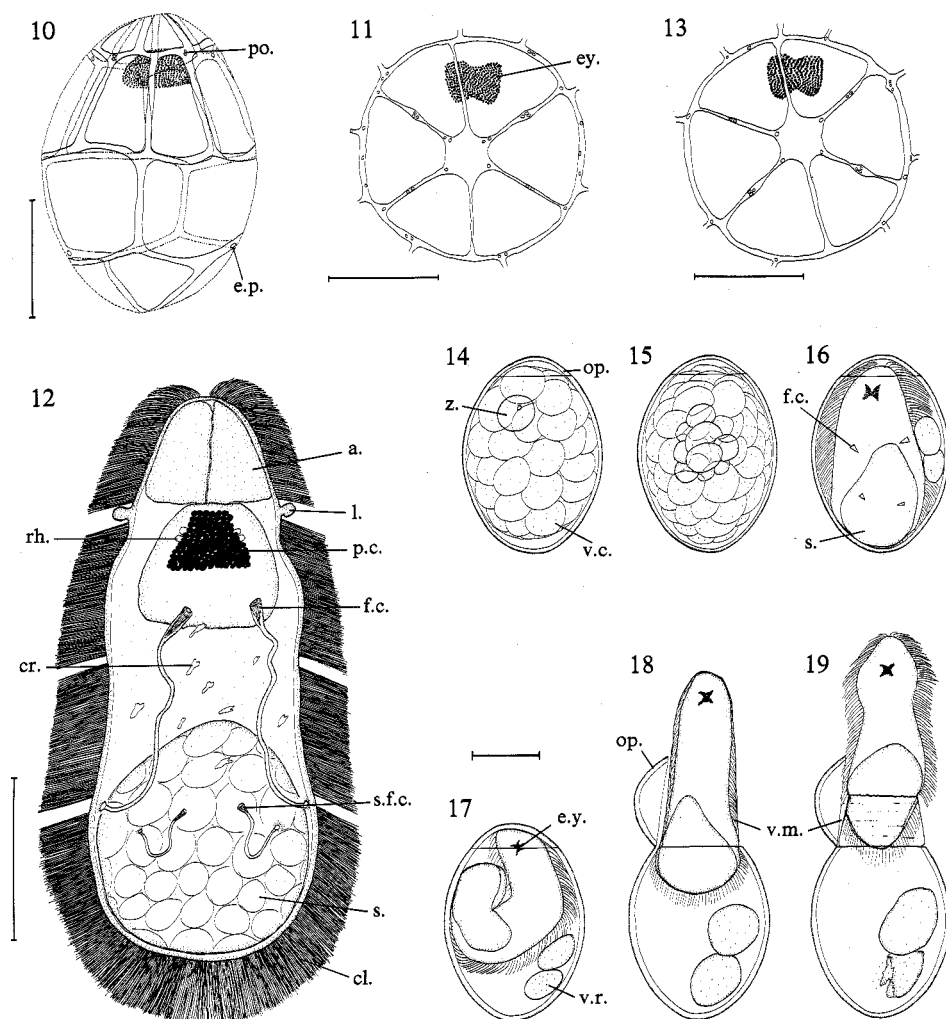
### Life Cycle Studies

Although sufficiently different to warrant generic differentiation, the worms are quite closely related and the details of their life cycles are presented together for economy. Intramolluscan stages of both worms are quite similar, and pertinent differences are highlighted by italicization.

# Miracidia

## (i) Description (Figs 10–13)

Ovoid to spheroid. Cilia on 4 tiers of epidermal plates (Fig. 10), with formula 6 : 9 : 4 : 2. Tier I, the most anterior, with 6 triangular plates (2 subdorsal, 2 lateral, 2 subventral) with posterior margins at level of eyespots; tier II with 9 rectangular plates (2 dorsolateral, 2 lateral, 2 ventrolateral, 2 subventral, 1 ventral); tier III with 4 trapezoidal plates (2 dorsolateral, 2 ventrolateral); tier IV with 2 helmet-shaped plates (1 dorsolateral, 1 ventrolateral). A lappet on left and right sides, visible only in living specimens (Fig. 12). Pattern of apical pores differs between *T. blepta* (Fig. 11) and *S. callista* (Fig. 13).



**Figs 10–13.** Miracidium. 10–12, *T. blepta*: 10, epidermal plates; 11, en face view; 12, internal features. 13, *S. callista*, en face view.

**Figs 14–19.** Development and hatching of eggs of *T. blepta*: 14, miracidium soon after touching snail; 15, egg with developing miracidial embryo; 16, egg with fully developed, active miracidium; 17, miracidium pressing on egg operculum; 18, miracidium ejected within vitelline membrane; 19, miracidium piercing vitelline membrane.

For abbreviations, see p. 180. Scale lines, 25  $\mu$ m.

Internally, indistinguishable, each having two concave pigment cups, joined medially to form an hourglass shape, each with two visible rhabdomeres. 2 large flame cells opening through ducts on right and left sides, just anterior to posterior most row of epidermal plates. Flame cell nuclei the only nuclei visible in body of miracidium, except those of developing sporocyst, even when lacto-orcein used to stain cell nuclei.

In posterior half of miracidia, motile, spheroidal developing sporocysts have 2 small active flame cells opening laterally through ducts. Apical end of the miracidia with 2 longitudinally arranged gland cells, followed posteriorly by clear area with eyespots; between midbody and sporocyst are many small crystalline bodies, less numerous at level of sporocyst.

#### (ii) Comments

There are very few descriptions of lepecreadiid miracidia. The miracidium of *Lepocreadium album* is conical, with two projections from the apical end formed by the ducts of two claviform gland cells. The epidermis has a few ciliated polyhedral cells. It has two flame cells, with ducts symmetrical about the midline, which open together, just anterior to the midbody (Palombi 1937). Palombi did not describe eyespots.

Macfarlane's (1951) description of the miracidium of *S. anguillae* is more complete. He illustrates two longitudinally arranged apical gland cells. Although he describes five rows of ciliated epidermal cells, he did not count the number of cells per row. He describes two flame cells, but although he described an active germinal mass which he assumed to be a redia within the hindbody of the miracidium, he did not observe any active flame cells or other structures within it.

#### (iii) Development

The zygote of the newly laid egg is a large clear cell located near the opercular end. The rest of the egg is filled with large, granular vitelline cells (Fig. 14). As the embryo develops the vitelline cells become more granular, and within 2 days their opacity limits study of the developing embryo (Fig. 15). Intact vitelline cells persist in the abopercular end of the egg for several days. Vitelline cell remnants form large refractile vacuoles at the ends of the egg. By day 5, at 20–25°C, most miracidia of *T. bleptae* have developed some eye pigment and at day 6 most show active flame cells. Development continues rapidly and by day 7 miracidia are fully developed and active (Fig. 16). *Development of S. callista is about 1 day slower at 20–25°C, and 2 or 3 days slower at 10–15°C.* By then the developing sporocyst within the posterior half of the miracidium shows flame-cell movement and is capable of contraction and extension independent of the miracidium.

#### (iv) Hatching of the egg

At temperatures of 20–25°C and 10–20°C, respectively, *T. bleptae* begins to hatch 7 and 14 days after the eggs are laid. *S. callista* eggs begin to hatch on the *ninth day at 15–20°C and the 11th–14th day at 10–15°C.* Most eggs hatch in the first hours of daylight, and light stimulates the activity of the miracidium within a developed egg; very few eggs hatch in complete darkness.

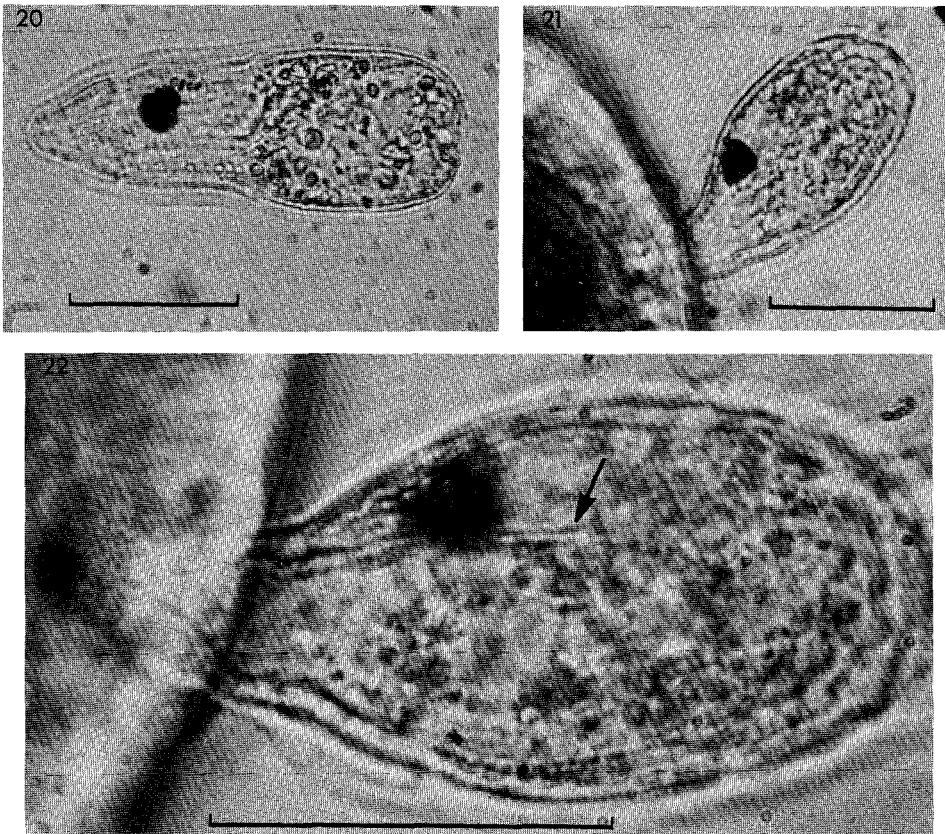
Several hours before hatching, the miracidium begins to slowly contract and stretch. By an hour before hatching, it begins to press on the operculum about every 30 s, during which vigorous ciliary activity occurs. It starts to continuously probe the operculum, as though seeking a weak point or applying a digestive enzyme (Fig. 17), and occasionally it rapidly contracts and extends its body.

No viscous cushion is ever visible between the operculum and the miracidium. Flame-cell activity increases, as the vitelline remnants enlarge and occasionally burst. Some miracidia rotate on their longitudinal axis immediately before hatching.

The miracidium continues to contact the operculum until the latter swings open rapidly. The miracidium does not attempt to protect its apical end. As its cilia quivers, the

miracidium, still enclosed in the vitelline membrane, is quickly ejected for three-quarters of its length out of the shell, but is stopped when the vitelline membrane fails to pass completely through the opercular opening (Fig. 18). Seconds later, the miracidium pierces the membrane and swims away (Fig. 19).

When the active miracidium presses on the operculum it may be applying digestive enzymes to the opercular cement, or it may be damaging the vitelline membrane to allow an influx of water, or both. Rowan (1956) reported that the miracidium of *Fasciola hepatica* releases a hatching enzyme which digests the opercular seal. Wilson (1968) believed that no such enzyme exists, but that the activity of the miracidium only damages the vitelline membrane, allowing water to enter.



Figs 20–22. Miracidium of *T. blepata*; 20, swimming; 21, touching snail; 22, sporocyst elongating within and moving anteriorad. Scale lines, 25  $\mu$ m.

Eggs have no viscous cushion or pad under the operculum, such as Pearson (1956) and Rowan (1957) have observed in other trematodes.

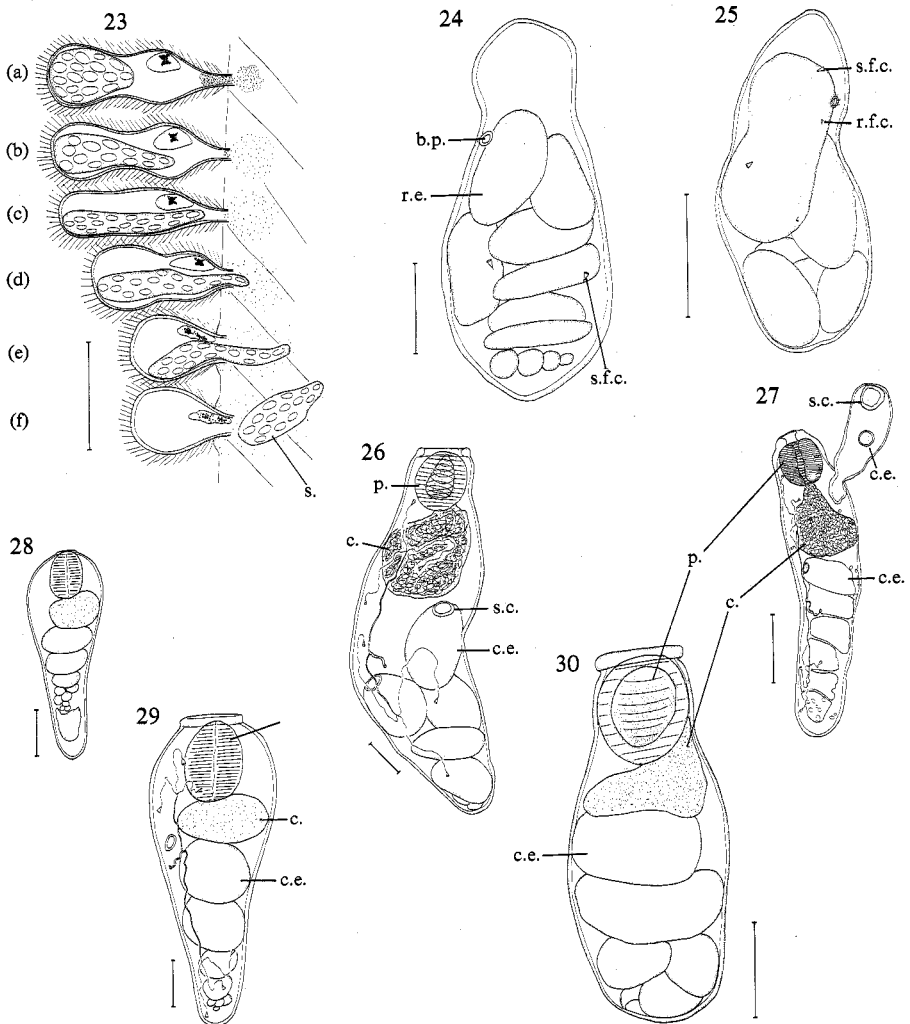
The increases in flame-cell activity and the swelling of vitelline remnants before hatching indicates that osmosis is occurring. The resulting osmotic pressure causes the ejection of the miracidium and the subsequent rupture of the vitelline remnants. Rowan (1957) measured an increase in water within the egg as hatching neared.

The above authors and others have presented strong evidence that the miracidium is ejected, sometimes even backward or when dead, if the eggs are in a hypo-osmotic solution. Kearn (1975) reported that the hatch of the oncomiracidium of *Entobdella soleae*, a

monogenean, does not depend on osmolarity but follows the application of enzymes and pressure by the oncomiracidium on the operculum.

Kassim and Gilbertson (1976) found that the osmolarity of the medium affects the rupture of the anoperculate eggs of *Schistosoma mansoni*. Eggs hatch if the ion concentration in the medium allows an influx of water which swells and ruptures the egg, expelling the miracidium even if it is dead.

The development and hatching of lepecoreadiid eggs have seldom been reported. Stunkard (1969) found that eggs of *Neopechona pyriforme* hatch in 9–10 days at laboratory temperatures. Macfarlane (1951) found that eggs of *S. anguillae* hatch by contractions and



**Fig. 23.** Penetration of snail by sporocyst of *T. blepta*: (a) miracidium soon after touching snail; (b) sporocyst beginning to elongate; (c) moving anteriorad; (d) beginning to leave miracidium; (e) entering snail; (f) within snail tissue.

**Figs 24, 25.** Sporocysts 2 weeks after penetration: 24, *T. blepta*; 25, *S. callista*.

**Figs 26, 27.** Redia, showing internal features, of: 26, *T. blepta*; 27, *S. callista*.

**Figs 28–30.** Developing rediae: 28, *T. blepta*, 2 weeks after penetration; 29, *T. blepta* after 4 weeks; 30, *S. callista* after 4 weeks.

For abbreviations, see p. 180. Scale lines: 23–27, 30, 50  $\mu$ m; 28, 29, 25  $\mu$ m.

extensions of the body as it pushes up the operculum, rather than through the motile power of the beating cilia. He did not report the period of development.

(v) *Swimming and penetration of the first intermediate host*

A swimming miracidium is usually clavate or fusiform (Fig. 20) but contracts to a spheroid when it stops. It swims with its apical end forward and, although it swims generally in a straight path, its body moves through a tight counterclockwise helix while rotating slowly counterclockwise on its longitudinal axis. It shows positive phototaxis.

When it chances to swim within approximately 5 mm of a *P. brazieri* it makes several rapid loops and sometimes becomes trapped in the snail's mucus. If the miracidium touches the host, its anterior end becomes fastened to the snail's tissue (Figs 21, 23a). Its cilia remain very active, and the body contracts and extends for about the first 5 min, during which time the anterior end stretches into a cone, which penetrates just into the tissue of the snail.

Within minutes, the sporocyst within the miracidium becomes active, and extends to just anterior to the miracidial eyespots (Figs 23b, 23c). The sporocyst continues to stretch and probe until it begins to pass out of the miracidium into the snail, often swelling the surrounding snail tissue externally as it does so (Figs 22, 23d, 23e). The miracidium contracts as the sporocyst penetrates further into the snail, the body of the miracidium contracts into a spheroid, which is pulled tightly against the snail as though exerting internal pressure to aid the sporocyst to exit (Fig. 23f). The sporocyst then slips out of the miracidium into the snail's tissue, where it continues to extend and contract, moving out of sight within the snail's tissues.

The miracidium soon dies and the eyespots break up. At any time during penetration, slight pressure dislodges the miracidium. Snails being penetrated by several miracidia often withdraw into their shells, but this did not appear to hinder the penetration.

Cable (1972) and others have discussed the behaviour of miracidia. They reported that some miracidia respond to the snail's presence by swimming faster and in tight circles, because they detect some substance which diffuses from the snail into the surrounding water. Miracidia of *T. blepta* often try to penetrate a glass surface over which snails have just passed, as if some identifiable and attractive trace of the snail, presumably in its mucus, had rubbed off onto the glass.

The behaviour of only one lepecreadiid miracidium has been reported, that of *S. anguillae* by Macfarlane (1951). He reported that the miracidium swam rapidly toward the light, often to the surface, revolving slowly on its long axis. Although he reported an active, semi-independent, intramiracidial body which he believed to be a redia, he did not describe penetration of the snail, which is likely to be a similar process to that in *T. blepta* and *S. callista*.

*Sporocysts*

(i) *Description* (Figs 24, 25)

Thin-walled, ovoidal; *T. blepta*, 202 (175–240, 4)  $\times$  85 (75–95, 4) (Fig. 24); *S. callista*, 141 (135–148, 2)  $\times$  74 (64–84, 2) (Fig. 25). Anterior end usually pointed; although this end appears quite dense, no pharynx observable. No caecum observed. Excretory system, 2 flame-cells just posterior to midbody, one on each side of body, each opening on lateral surface through a duct. A lateral, circular birthpore.

At 4 weeks after penetration, sporocyst contains 6 or 7 ovoidal developing rediae; largest 38 (31–46, 2)  $\times$  32 (31–33, 2) in *T. blepta*; 48 (44–52, 2)  $\times$  40 (36–44, 2) in *S. callista*.

(ii) *Development*

In the newly hatched miracidium, the sporocyst is an ovoidal mass (Fig. 12) with 34 (30–41, 8) cells arranged so that the centre appears less dense. The sporocyst elongates as it penetrates the snail and becomes pointed anteriorly (Fig. 23). At 2 weeks after penetration,



the sporocyst is found in the anterior body of the snail near the heart. It has grown to four times its size at penetration, and contains germinal bodies which will become the rediae. At this time a lateral birth pore is first observed. By 4 weeks, the sporocyst has further enlarged and now contains six or seven rediae, but still has only two flame-cells as it did within the miracidium. No germinal bodies are now observed and there is no persistent germinal mass as defined by Cort *et al.* (1954). After 4 weeks the sporocyst is no longer observed and is assumed to have died. *S. callista* develops faster, has a wider size range of developing rediae within it and becomes senile faster.

None of the previous studies of lepecreadiids have described the penetration of the snail, and therefore it is not known whether the miracidium of other species enters the snail or injects a sporocyst or a redia. In only two studies was a sporocyst described (Stunkard 1969; Lengy and Shchory 1970), but all authors describe a redial stage.

Macfarlane (1951) did not find a sporocyst of *S. anguillae* but assumed that if it did exist it would disappear soon after a single brood of rediae had been liberated. The germinal body he described and illustrated within the miracidium of *S. anguillae* lacked any trace of a pharynx or a caecum, and it is likely that this is a sporocyst which penetrates a snail as does that of *S. callista*. It would then quickly produce rediae and die shortly afterward.

## Redia

### (i) Description (Figs 26, 27)

Described from snails infected in nature.

*T. blepta*: Muscular, sac-like, 192 (143–298, 20)  $\times$  67 (35–91, 20), with a well developed terminal sphincter muscle directly anterior to a muscular, ovoidal pharynx 27 (22–36, 20)  $\times$  31 (24–38, 20) (Fig. 26). Pharynx connected directly to large, conspicuous, ovoidal caecum, 29 (14–81, 20)  $\times$  42 (19–81, 20) filled with granular orange material from digestive gland of host snail. 3 (1–6, 20) spheroidal germinal bodies (developing cercariae), the largest 48 (17–94, 20)  $\times$  38 (17–62, 20), located just posterior to caecum, lateral to birthpore. No trace of eye pigment. Flame-cell pattern 2 (3 + 3); excretory pores lateral to midline, one-third of body length from posterior end (Fig. 26).

*S. callista* (Fig. 27): Similar to that of *T. blepta*, 245 (165–314, 15)  $\times$  80 (52–111, 15). Muscular pharynx circular, 26 (17–37, 15), connected to ovoid caecum, 33 (14–58, 14)  $\times$  20 (9–30, 14), filled with snail digestive gland tissue. 5 (3–9, 8) germinal bodies, the anterior the most developed, 53 (39–70, 10)  $\times$  60 (27–72, 10), located lateral to birthpore, just posterior to caecum. No eye pigment. Flame-cell pattern 2 (3 + 3), excretory pores laterally placed about halfway along the body length.

### (ii) Comments

The redia of *S. callista* is generally larger than that of *T. blepta*. This is not surprising, as the germinal bodies which the redia of *S. callista* contains, and the cercariae these develop into, are larger.

It is common for the rediae of species in the family Lepocreadiidae to be given only brief, general descriptions. Often more than one generation has been recognized, but these are designated only as large and small rediae.

All lepecreadiid rediae reported in the literature are longer than the two species in the present study. That of *Cercaria levantina* 2 is 1100  $\mu$ m (Lengy and Shchory 1970), *Lepocreadium pegorchis* is over 1000  $\mu$ m (Bartoli 1967), and that of *Lepocreadium areolatum* is up to 1600  $\mu$ m (Stunkard 1980a). These lengths are 4–6 times those of the rediae of *T. blepta* and *S. callista*, which may be attributable to the comparatively small size of their snail host, *Posticobia* (2–3 mm).

The mature redia of *S. anguillae* is more than three times longer than that of *S. callista* or

*T. blepta*, but is much thinner at only one-quarter the width. *S. anguillae* contains 10–25 immature cercariae, more than either *T. blepta* or *S. callista*; its caecum is also longer and thinner. Macfarlane (1951) also observed ten flame-cells per side in *S. anguillae*, compared with six in *T. blepta* and *S. callista*.

### (iii) Development

Rediae are found in the digestive gland of *Posticobia* within 2 weeks of the sporocyst penetrating the snail. Sometimes germinal bodies are visible within the rediae (Fig. 28). At 4 weeks, rediae are still in the digestive gland. They have grown, and the pharynx is enlarged and is now oval in *T. blepta* (Fig. 29) and circular in *S. callista* (Fig. 30). The oval caecum is full of snail tissue. There are from three to six ovoidal germinal bodies within the redia, but no longer a posterior mass of germinal cells. At 4 weeks, much smaller rediae, believed to be of the second generation, are observed. These are very similar to the previous generation and can be separated only by their small size. Samples of infections of different ages showed little or no increase in the average size of the rediae with time. This may be due to the continuing birth of rediae, at the same time as older, larger ones are dying.

By 6 weeks after penetration of the snail, the continuing production of rediae results in a large range of sizes, so that the ages of developing rediae can no longer be determined.

Immature cercariae of *T. blepta* first appear within the snail at 11 weeks at 15–20°C. Some or all of the redial 'generations' may then produce cercariae.

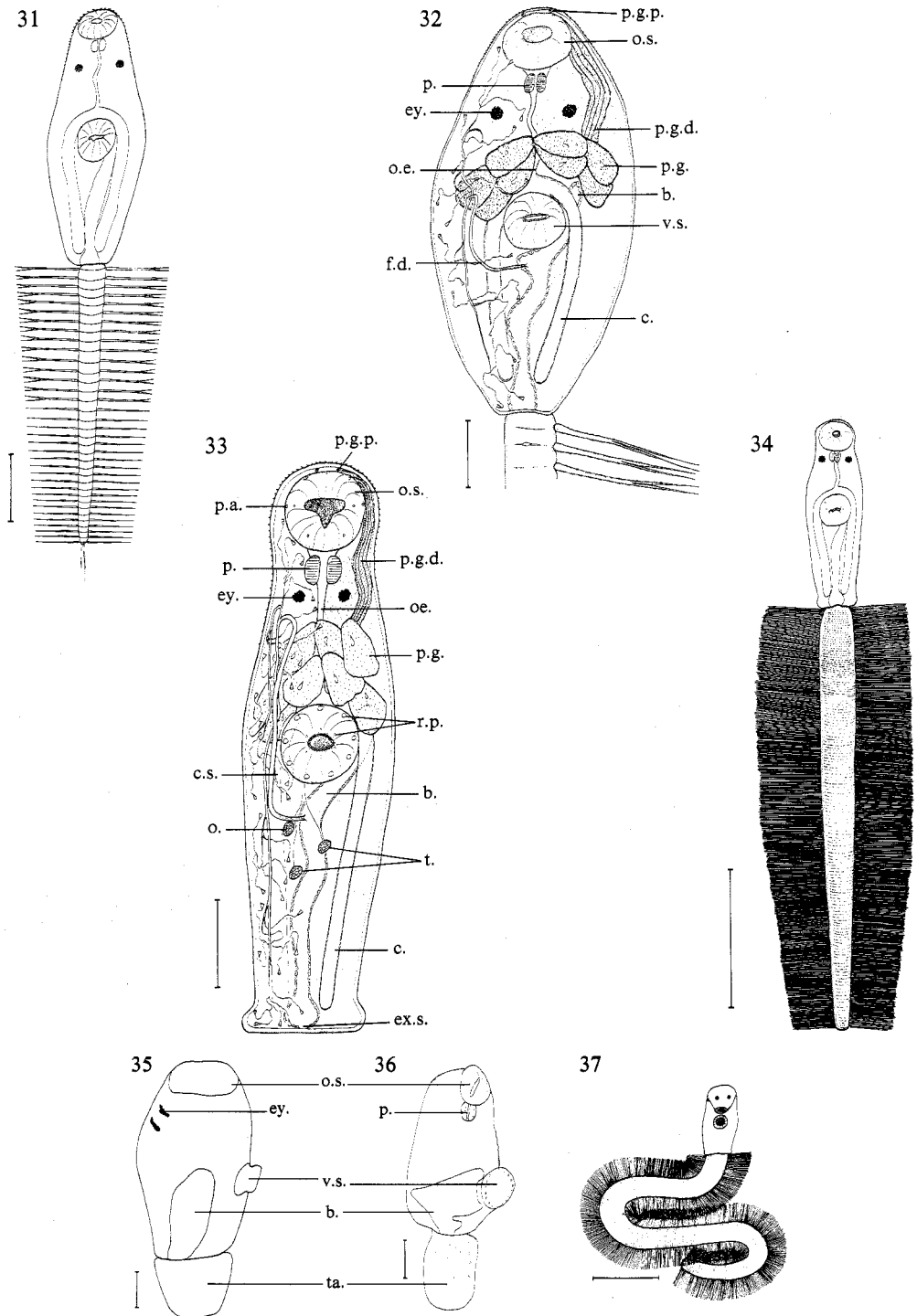
## Cercaria

### (i) Description (Figs 31–41)

Based on observations of live material supplemented by measurements of fixed specimens.

*T. blepta*: Distomate, pharyngeate, ophthalmitrichocercous (Fig. 31). Body spinose, flattened ovoid, 186 (116–215, 11) × 76 (62–86, 11). Oral sucker large, ovoidal, subterminal, ventral, 37 (32–44, 11) × 35 (30–41, 11). Ventral sucker large, circular 34 (30–41, 11) with papillae. Tail plumose, slender, cylindrical, setiferous laterally, length 212 (170–252, 11), widest at anterior end 18 (13–22, 11). Setae serrated, blade-like (Fig. 32), maximum length 52 (44–60, 11) at midtail; 35 setae per side and 2 or 3 terminal, anterior 15 pairs bifurcate, others simple. Few large dentiform scales on dorsal and ventral surface. Prepharynx very short; pharynx oval, 13 (9–16, 11) × 15 (10–19, 11); oesophagus slender, tube-like, length 39 (9–16, 11), bifurcation just anterior to ventral sucker; caeca thin-walled, end blindly at posterior end, length 87 (70–104, 11) (Fig. 32). Penetration glands lacrimiform, 8 arranged in 2 groups of 4, anterior and lateral to ventral sucker, 4 swollen ducts from each group passing forward lateral to eyespots, and opening through diamond-shaped bundle of 4 pores on dorsal rim of oral sucker. Cystogenous glands small, spherical, numerous, near dorsal surface, between oral and ventral suckers. Rudiments of testes and ovary rarely present. Cirrus sac not visible. Flame-cell formula 2[(3 + 3 + 3)(3 + 3 + 3)]; anterior and posterior collecting ducts joining main ducts anterolateral to ventral sucker; main ducts, with series of flagella, joining excretory bladder just posterior to ventral sucker. Bladder tubular, 95 (81–116, 11) × 23 (17–29, 11), size varying with content, extending from lateral to ventral sucker to posterior end; wall epidermal, thin; excretory pore at tail-body junction. Eyespots prominent, length 7, width 9, 2 'lenses' each.

*S. callista*: Distomate, pharyngeate, ophthalmitrichocercous (Fig. 33). Body spinose (Fig. 41), cylindrical (Fig. 38), length 264 (254–288, 12), widest at ventral sucker 75 (59–99, 12). Oral sucker large, ovoidal, subterminal, ventral, 39 (32–47, 12) × 44 (37–52, 12); with 8 papillae (Fig. 33). Ventral sucker large, circular 43 (37–52, 12); with 2 concentric rings of papillae, inner of 5 small and pointed papillae, outer of 9 large and blunt (Fig. 40). Tail very large, plumose, cylindrical, setiferous laterally (Fig. 34), length 610 (464–731, 9), widest at midlength 65 (40–86, 9). Setae maximum length 103 (89–116, 9) at midtail, width 1;



**Figs 31–37.** Cercaria: 31, 32, *T. blepta*, entire and body only; 33, 34, *S. callista*, body only and entire; 35, 36, developing *S. callista* (35) and *T. blepta* (36); 37, swimming *S. callista*. For abbreviations, see p. 180. Scale lines: 31, 33, 50  $\mu$ m; 32, 35, 36, 25  $\mu$ m; 34, 200  $\mu$ m; 37, 100  $\mu$ m.

1140–2000 per side on lateral surface in 190–250 rows; setae becoming transverse rows of dentiform scales on dorsal and ventral tail surface (Fig. 39). Prepharynx very short (Fig. 33); pharynx 14 (12–19, 12)  $\times$  17 (11–23, 12); oesophagus slender, length 80 (60–100, 12), bifurcation just anterior to ventral sucker; caeca thin-walled, ending blindly at posterior end, length 30 (20–40, 12). Penetration glands lacrimiform, 8 arranged in 2 groups of 4, anterior and lateral to ventral sucker, 4 swollen ducts from each group passing forward lateral to eyespots and opening through diamond-shaped bundle of 4 pores on dorsal rim of oral sucker. Cystogenous glands small, spherical, numerous, near dorsal surface between oral and ventral suckers. Testes 2, oblique; anterior testis oval, 5  $\times$  7, sinistral; posterior testis circular, 6. Ovary oval, 9  $\times$  8, anterior to testes, dextral. Cirrus sac poorly developed, curved, 27  $\times$  12, dextral to ventral sucker. Flame-cell formula 2[(4 + 5 + 5)(5 + 5 + 5)], somewhat variable; anterior and posterior collecting ducts joining main ducts at anterior border of penetration glands; main ducts with c. 10 long flagella in lumen, joining excretory bladder just posterior to ventral sucker. Bladder tubular, 96 (86–114, 12)  $\times$  22 (5–46, 12), extending from just anterior to ventral sucker to posterior end; wall epidermal, thin; excretory pore at tail-body junction. Eyespots 2, prominent, circular, 9, 2 'lenses' each, with mouth of pigment cup directed anterolaterally.

#### (ii) Comments

The cercariae of *T. blepta* and *S. callista* differ in several respects. That of *T. blepta* is a flattened ovoid, whereas that of *S. callista* is cylindrical; the tails are also different. The flame-cell pattern of *T. blepta* is 2[(3 + 3 + 3)(3 + 3 + 3)] whereas that of *S. callista* is 2[(4 + 5 + 5)(5 + 5 + 5)].

The cercaria of *T. blepta* is similar to that of *S. anguillae* described by Macfarlane (1951). The tails, though similar, have different numbers of setae: *T. blepta* has 35 on each side and two or three terminally, whereas *S. anguillae* has 37 or 38 bristles on each side and one wider terminal bristle. The anterior 15 setae of *T. blepta* are bifurcate, but the anterior 20 or 25 of *S. anguillae* are paired. As the cercaria of *S. anguillae* is very similar to that of *T. blepta*, both differ from *S. callista* in the same ways.

A characteristic of lepopocreadiids is that their cercariae have tails bearing setae. In the genus *Lepocreadium* (e.g. *L. album*) these setae are in tufts of four or five, and not single as in *T. blepta* or partly single and partly paired as in *S. callista*. *N. pyriformis*, *Opechona bacillaris*, and *Holorchis pycnoporos* also have tufts of setae. Single setae, where present, are few, short and thin, and more sensory than locomotory. An example of such a cercaria is that of *Homalometron pallidum*, in the Homalometronidae, a family related to the Lepocreadiidae.

#### (iii) Development

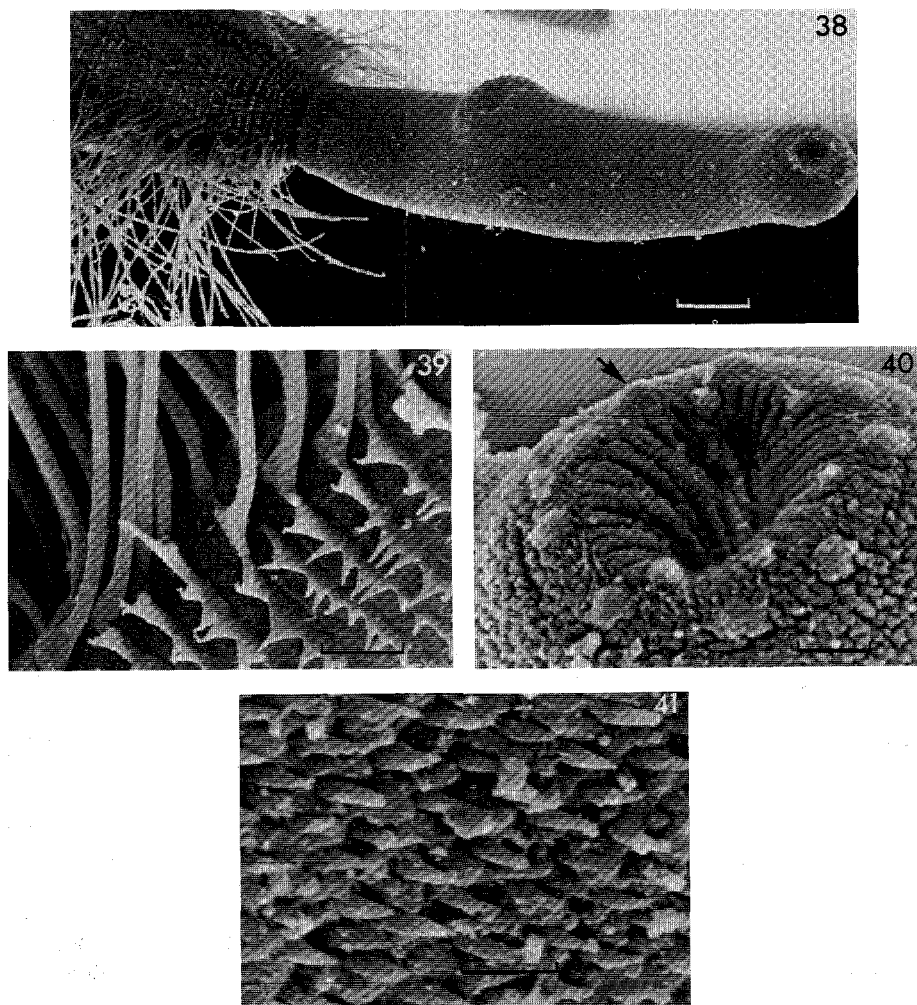
The germinal bodies which become cercariae form at the posterior end of rediae. Few features of even the most developed cercariae within the redia can be recognized. The ventral sucker is sometimes observed but tail setae are not. Eyespots are sometimes visible in cercariae of *S. callista* (Fig. 35) but not of those in *T. blepta*.

Outside the rediae, the least mature cercariae are found near them. These have an oral sucker, a pharynx, a protruding ventral sucker, a large bladder and a stumpy ovoidal tail with no visible setae (Fig. 36). As a cercaria develops, the tail and body elongate and setae are formed.

Mature cercariae are found near the surface of the snail's digestive glands approximately 2 weeks after the first are born. The first cercariae of *T. blepta* to emerge from the snails do so at 70 (40–84, 14) days at 15–20°C and at 85 (71–102, 18) days at 10–20°C after the snails are exposed to miracidia. The first cercariae of *S. callista* emerge at 59 (57–68, 6) days at 15–20°C and 89 (71–119, 64) days at 10–20°C.

Stunkard (1980a) reported that the cercaria of *Lepocreadium areolatum* matures in the

haemocoel, develops tail setae shortly before emergence, and emerges through the snail's gills. The emergence of the cercariae of *T. blepta* has not yet been observed, but the gills may be the route in this species also.



**Figs 38–41.** Cercaria of *S. callista*: 38, body; 39, tail setae; 40, ventral sucker, arrow indicates papillae; 41, body spines. Scale lines: 38, 20  $\mu\text{m}$ ; 39, 4  $\mu\text{m}$ ; 40, 41, 2  $\mu\text{m}$ .

#### (iv) Behaviour and swimming

Most cercariae of *T. blepta* emerge at dusk and show a weak but positive phototaxis. Changing the water the snails are kept in stimulates the emergence. The cercaria swims tail first by generating a sigmoidal curve at the tip of its tail, which it sweeps toward its body, the anterior end of which is bent toward the ventral sucker. The cercaria moves in a clockwise helix through the water, usually near the bottom. A newly emerged cercaria swims almost constantly, stopping briefly when it contacts the surface, to extend its body and tail. Sometimes the setae are spread by pulling the tail across the rim of the ventral sucker. As a cercaria ages it spends more and more time in its resting position, lying dorsal side down on

the bottom, body arched upward and tail outstretched, sometimes making weak swimming motions.

Most cercariae of *S. callista* (Fig. 37) emerge at dawn and are active during the day, when they are highly visible to feeding fish. These cercariae do not survive overnight, but have the longest possible period of daylight to attract and be eaten by a fish host. Cable (1972) reported correlations, from his own work and that of others, between the time when cercariae emerge and the habits of the next host; cercariae emerge when the host is most likely to be contacted, normally when it is most active.

Bright light and changing the snail's water stimulate emergence of *S. callista* cercariae.

Normally, any directional movement of the cercaria of *S. callista* is upward in a tight helix. This cercaria, unlike that of *T. blepta*, does not usually move much laterally or vertically during its brief periods of swimming (usually less than 10 s) and spends longer periods in its resting position hanging obliquely, body down, ventral side up and tail outstretched. Occasionally the cercaria may coil its tail into a tight spiral.

Cercariae of *T. blepta* and *S. callista* swim for 6–8 h. After this, cercariae of *T. blepta* usually lose their tails and crawl, leech-like, along the bottom for several more hours. They remain infective even after they lose their tails, but the chance of contact with a host is reduced. As the cercaria ages, it spends less and less time swimming, and during its prolonged resting periods it may sink until its dorsal body surface touches the bottom. Cercariae of *T. blepta* and *S. callista* remain active and alive for less than 8 h in pond water, but 1 day in 0.8% saline.

The intermittent swimming of the cercaria may allow it to survive longer than if it were constantly swimming, by reducing its energy expenditure and thus increasing its chances of contact with a fish host.

The pattern of emergence of lepecreadiids varies; for instance, the cercaria of *Lepocreadium setiferoides* emerges by day or by night, and is photonegative (Martin 1938). Most cercariae of *N. pyriforme* emerge during the night or early morning and are photonegative but become photopositive (Stunkard 1969).

The action of pulling the tail across the edge of the ventral sucker is similar to the tail-cleaning motion reported by Køie (1975) for the cercaria of *O. bacillaris*, another lepecreadiid.

The cercaria of *S. anguillae* crawls along the bottom, but when it does swim it does so continuously, in a series of waves that cause it to move horizontally (Macfarlane 1952); it does not have a marked positive phototropism.

It would be advantageous for *T. blepta* cercariae to emerge at night, because their movements near the bottom would bring them near the nocturnally less active tadpoles and fish which they infect. Contact with an active host would be too fleeting for attachment, the prelude to penetration, to take place.

#### (v) Penetration of the second intermediate host

The cercariae of *T. blepta* are rarely accidentally or deliberately swallowed, as are those of *S. callista*. When the cercaria of *T. blepta* contacts a tadpole it immediately elongates its anterior end and attaches to the skin, holding its tail in a slight arc nearly parallel to the tadpole's surface. The cercaria probes the tadpole's skin continuously with its anterior end, apparently easily dissolving it, and then enters a shallow channel under the skin. Setae may fall off, but the tail of the cercaria does not drop off until it is the only part of the cercaria remaining outside. Penetration usually takes less than 5 min.

Cercariae of *T. blepta* penetrate anywhere on the surface of a tadpole, even into the thin, transparent tail fins, but most heavily at the junction of the body and tail. In fish, the bases of the fins are the most heavily penetrated area. These cercariae may occasionally be eaten by smaller fish or accidentally inhaled by fish or tadpoles and swallowed. They may then penetrate the throat region or intestine and encyst in the surrounding tissues.

Smaller fish eat the cercariae of *S. callista*, and larger fish draw them into their mouths with the water which traverses the gills. A slight mechanical shock will dislodge the large tails of these cercariae, and those which are inhaled usually lose their tails as they strike the gill rakers, the tails passing out through the operculum while the bodies attach inside the gill chamber.

The cercariae of *S. callista* may also penetrate the skin. The process of penetration takes about 20 min. A cercaria on the bottom, which has lost its tail, may remain infective for several hours, but as with *T. blepta* its chances of contacting the host are reduced.

Stunkard (1969, 1980a) found that the cercariae of *N. pyriforme* and *Lepocreadium areolatum* use their tails to attach to a host initially; later they penetrate the host, leaving behind their tails, which swim by themselves for hours. Cercariae of *T. blepta* were not observed to attach to hosts by their tails, but the latter did sometimes become stuck to mucus on the surface of a fish.

Macfarlane (1952) believed that cercariae of *S. anguillae* attacked gobiids from below or from the sides while they lay in the shadows or interstices of a shingle bed. This cercaria is very similar in morphology to *T. blepta*, which attacks fish and tadpoles as they lie on the bottom.

### *Metacercaria*

#### (i) *Description* (Figs 42–50)

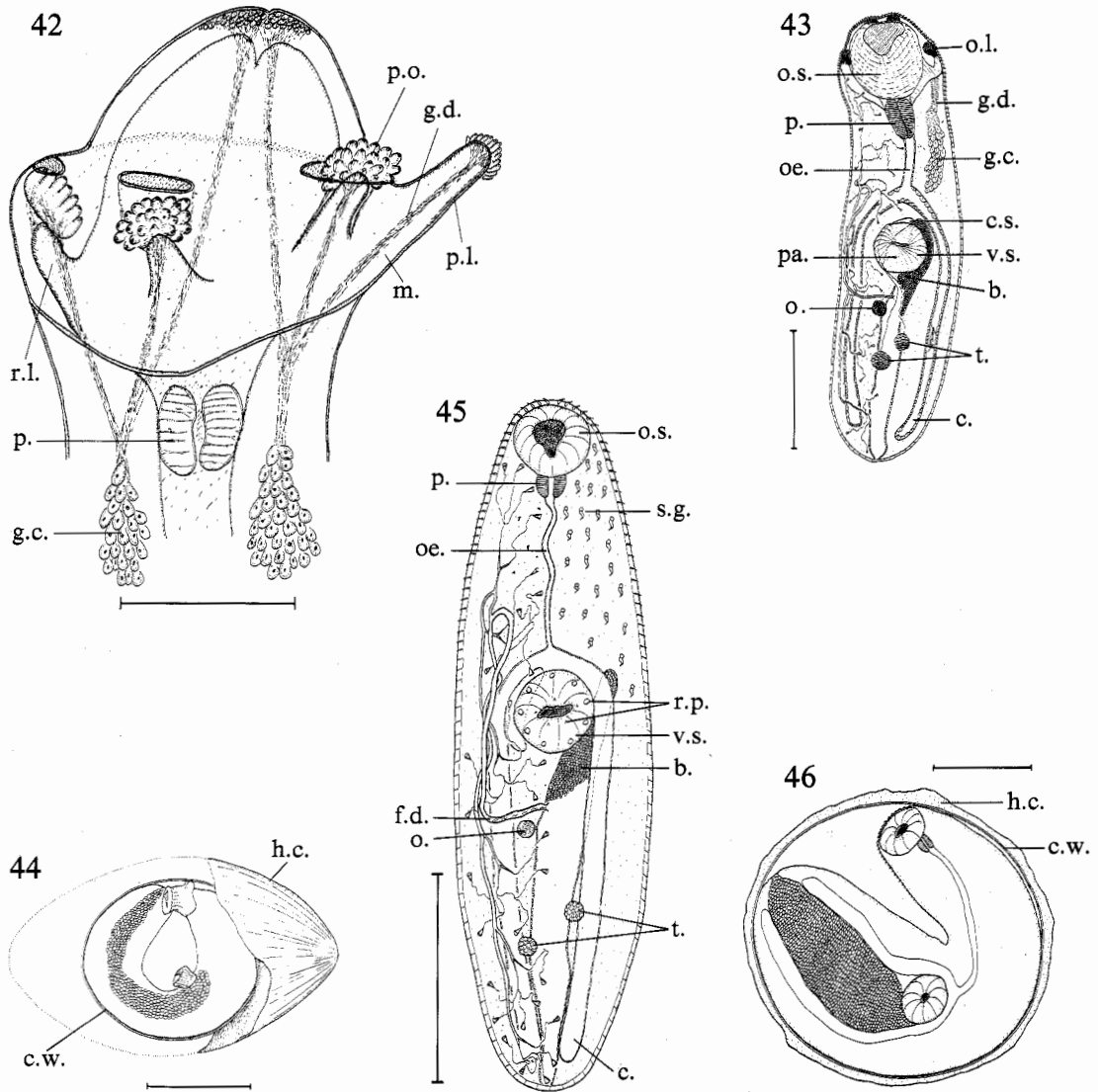
*T. blepta*: Based on observation of live material supplemented by measurement of 10 fixed specimens from dorsal muscle of wild-caught *Gobiomorphus australis*; description of cyst wall and host capsule based on 4 live cysts from pharyngeal muscle of *Gobiomorphus* sp. infected experimentally 8 weeks previously.

Flattened elongate (Fig. 43); 409 (232–530, 10)  $\times$  133 (101–168, 10); anterior surface covered with scales which become spines posteriorly extending to level of ovary and sparsely to posterior end. Oral sucker large, subterminal, ventral, retractable, circular, 80 (54–101, 10), glandular tissue on dorsal edge. Oral sucker lobes, 4 extensible with conical ampullae on ends, in a transverse row just dorsal to oral sucker margin (Figs 42, 47–50; see section following). Ventral sucker smaller than oral sucker, circular; 63 (44–77, 10); with 3 small, pointed, evenly spaced papillae around rim. Prepharynx very short; pharynx ovoidal, 23 (15–30, 10)  $\times$  31 (22–42, 10); oesophagus short, length 72 (37–136, 10), bifurcation anterior to ventral sucker; caeca ending blindly near posterior end, length 186 (106–259, 10)  $\times$  19 (12–25, 10), often filled with acicular crystalline bodies. Testes 2, equal, circular, 22 (17–30, 10), oblique, anterior testis sinistral, posterior to ovary; sperm ducts separate, joining at posterior end of cirrus sac; cirrus sac botuliform, extending from posterior margin of ventral sucker dextrally to just anterior, 64  $\times$  12. Ovary circular, 12, dextral, just posterior to ventral sucker. Eggs not present. Vitellaria poorly developed. Flame-cell formula 2[(3+3+3)(3+3+3)]; anterior and posterior collecting ducts joining main ducts anterolateral to ventral sucker; main ducts joining excretory bladder posterior to ventral sucker. Bladder tubular, full of refractile concretions; 207 (94–260, 10)  $\times$  19 (7–32, 10); extending from anterior to ventral sucker, dorsal to ovary and testes to the posterior end of body. Eyespot pigment scattered to forebody. Cyst spherical (Fig. 44), large, 320 (270–381, 4); cyst wall transparent, elastic, thin (4); host capsule ellipsoidal (spherical in tadpoles), 595 (577–614, 4)  $\times$  422 (381–456, 4); capsule wall fibrous, white, translucent, thickest at capsule ends (in ellipsoidal cysts), 129 (93–186, 8). Both host capsule and cyst wall easily removed.

Encysted *T. blepta* found in pharyngeal muscle, viscera and muscle at base of fins in *Hypseleotris galii*, *Gobiomorphus australis*, *Gobiomorphus* sp., *Mixophyes* sp. (tadpole) and *Litoria lesueri* (tadpole, natural and experimental infections).

*S. callista*: based on observation of live material supplemented by measurement of 15 fixed specimens from liver of wild *Retropinna semoni*; description of cyst, cyst wall and host

capsule based on 11 live cysts from pharyngeal muscle of *Nematocentris fluviatilis* infected experimentally 4 weeks previously.



**Figs 42-46.** Metacercaria. 42-44, *T. blepta*: 42, oral sucker lobes, dorsal view, in various stages of protraction; 43, whole body, showing only the anterior concretions in the excretory bladder; 44, cyst. 45, 46, *S. callista*: 45, whole body, as in Fig. 43; 46, cyst. For abbreviations, see p. 180. Scale lines: 42, 25  $\mu$ m; 43, 45, 150  $\mu$ m; 44, 200  $\mu$ m; 46, 75  $\mu$ m.

Flattened fusiform (Fig. 45), 334 (255-465, 15)  $\times$  152 (62-161, 15), anterior surface covered with scales which become spines posteriorly extending to level of ovary and sparsely to posterior end (Fig. 45). Suckers subequal; oral sucker subterminal, ventral, retractible, subcircular, 42 (32-49, 15)  $\times$  45 (37-54, 15); ventral sucker circular, 40 (30-52, 15), with 2 concentric rings of papillae, inner of 5 small and pointed papillae and outer of 9 large and blunt; skin glands abundant anterior to ventral sucker. Prepharynx very short; pharynx circular, 18 (12-40, 15); oesophagus short, 75 (40-110, 15)  $\times$  8 (4-12, 15), bifurcation



anterior to ventral sucker; caeca ending blindly at posterior end,  $170 (135-270, 15) \times 9 (5-12, 15)$ . Testes 2, equal, circular,  $12 (7-17, 15)$ , oblique, posterior to ovary; anterior testis sinistral; cirrus sac dextral, botuliform, slightly longer than diameter of ventral sucker,  $44 \times 15$ . Ovary circular, 7, seldom differentiated from primordium of female system. Egg not present. Vitellaria not fully developed. Flame-cell formula  $2[(4+5+5)(5+5+5)]$ ; anterior and posterior collecting ducts joining main ducts anterolateral to ventral sucker; main ducts joining excretory bladder anterior to ovary. Bladder tubular, may fill with refractile concretions until nearly ovoidal;  $150 (100-290, 15) \times 90 (50-140, 15)$ ; extending from just anterior of ventral sucker dorsal to ovary and testes, to posterior end. Eyespot pigment scattered in older specimens. Cyst spheroidal (Fig. 46),  $204 (167-250, 11)$ ; cyst wall thin, transparent, elastic, 2; host capsule spheroidal, fibrous, white, translucent, thickness  $11 (4-15, 11)$ .

Encysted *S. callista* found in pharyngeal muscle, viscera and muscle at base of fins in *Retropinna semoni*, *Pseudomugil signifer* and *Ambassis* sp.

## (ii) Comments

The metacercaria of *T. blepta* develops into a larger worm than does that of *S. callista*. *T. blepta* has a larger oral than a ventral sucker whereas the suckers are equal and smaller in *S. callista*. *S. callista* does not have the oral sucker lobes of *T. blepta*. Even as a metacercaria *S. callista* has numerous skin glands, which are few in *T. blepta*.

There is a considerable range of development in lepopocreadiid metacercariae. Some species of lepopocreadiid metacercariae such as *S. anguillae* may be 'progenetic' and egg-producing (Macfarlane 1951), whereas others, such as *Lepocreadium pegorchis*, are little advanced from the cercaria (Bartoli 1967).

## (iii) Development

When the cercariae of *T. blepta* and *S. callista* begin to encyst, they probe in all directions as though seeking to create a space large enough for their cysts. The cercaria becomes dorsoventrally flattened and begins to move in a tight circle by following its posterior end. When it is motionless, the cyst material is presumably secreted, and when it rotates, its anterior end probes and smooths, moulding the cyst wall. The wall is added to between periods of rotation, and the plane of rotation alters so that a complete spherical wall is formed. This takes less than 10 min, but the cercaria continues to rotate within the cyst for several hours, although less actively than earlier. Cercariae have rarely encysted on the bottom of the container.

The newly formed cyst of *T. blepta* and *S. callista* is very thin ( $2 \mu\text{m}$ ), elastic and transparent. The swollen ducts from the penetration glands of the cercaria are depleted during penetration and encystment, but the bodies of the penetration glands are only slightly smaller than before. Within 1 day the pigment granules of the eyespots begin to break apart, and the surface of the cyst wall may have a granular, opaque appearance which is the start of the host capsule.

At 1 week after encystment the metacercariae of *T. blepta* and *S. callista* are not active but are undergoing development and some changes of morphology. The eyespots are further broken up, the bladder is rapidly filling up with granules, and the oral sucker is no longer circular as in the cercaria but is developing raised areas which will become the oral sucker lobes.

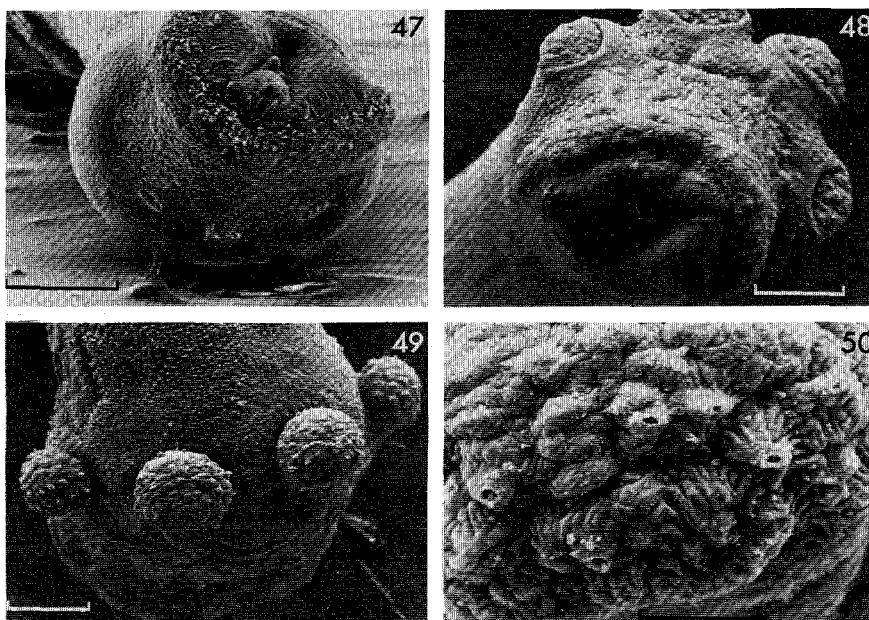
After 6 weeks the cyst of *T. blepta* does not become larger, but the metacercaria continues to develop and enlarge within the cyst. After 10 weeks the genitalia no longer enlarge or develop, possibly because the host capsule reduces the flow of nutrients to the metacercaria. Metacercariae of *T. blepta* are infective after 2 weeks of development, and most were alive 1 year after penetration of the cercariae in the laboratory.

Metacercariae of *S. callista* grow for about the first 2 weeks in the cyst, then stop growing. They are infective to eels within 7 days of encystment.

Many species of *Lepocreadium* have been reported to lie unencysted in their intermediate host. Stunkard (1980a) reported that the *L. areolatum* were unencysted in medusae and ctenophores. *Cercaria setifera*, probably *L. album*, has been found (Arvy 1953) with its tail still attached unencysted in the nudibranch *Phyllirhoe bucephala*. Arvy concludes that the host does not make a defence reaction to the cercaria.

### Oral Sucker Lobes of *T. blepta*

Four protractile lobes originate near the dorsal, external margin of the oral sucker (Figs 47–50). In the tip of each lobe, as well as the dorsal margin of the oral sucker, are several large polygonal ampullae containing granular material which stains light blue with Masson's trichrome stain, indicating that it contains a mucous or collagen compound. On the surface, each lobe is capped with conical projections, each with an opening at the apex, like a volcano



**Figs 47–50.** Oral sucker lobes of *T. blepta*; 47, fully retracted, upper surface ventral; 48, partly protracted; 49, protracted; 50, pores of ampullae on ends. Scale lines; 47–49, 40  $\mu\text{m}$ ; 50, 10  $\mu\text{m}$ .

(Fig. 50). There is apparently a cone for each of the large ampullae underneath. Under the ampullae, each lobe contains a column of muscle continuous with the musculature of the oral sucker; this can retract the ampullae caps until they are completely inside the anterior end of the worm and only slits reveal their location (Figs 42, 47). The ampullae, when fully retracted, are no longer globular (Fig. 49) but form a flat disc on the external face of the invagination (Fig. 42). The regions surrounding the lobes are less dense than the surrounding tissues, and contain connective tissue in addition to the gland ducts connecting the lobes to the gland cells in the forebody of the worm. When the lobes are protracted, the discoidal mass of ampullae is forced out by the lip of the elongating muscular oral sucker pressing externally on the ampullae, so that the outer lip of the invagination everts leaving the inner lip unmoved (Fig. 48). Although scales cover the anterior end of *T. blepta*, they are absent near the tips of the lobes and on the margins of the oral sucker.

From the caps of granule-containing ampullae on oral lobes, ducts pass backwards in two groups laterally, and connect with two large groups of nucleated cells which stain identically to the material in the ampullae. These gland cells fill most of the body between the pharynx and the cirrus sac and may be homologous with the frontal glands of other digeneans. These cells may produce the granular material, possibly a mucopolysaccharide, which the ducts carry to the ampullae and possibly also to the glandular tissue on the dorsal edge of the oral sucker. Material stored in the ampullae may act as a digestive enzyme. The lobes may also allow better attachment of this long cylindrical worm to the eel gut, which passes much coarse material such as snail shells, insect exoskeletons, and fish bones.

Yamaguti (1971) reported that three genera of lepecoreadiids in the subfamily Megalogniinae have oral sucker appendages or head-lobes. *Megalognia*, *Creptotrema* and *Creptotrematina* all have a pair of papilliform head-lobes anterodorsally on the oral sucker.

Some allocreadiids also have oral sucker lobes. It would appear that oral sucker lobes are not uncommon in these two families. No associated glands are described for any of the above, and in most the number and position of oral sucker lobes differ.

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