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The life cycle of *Hapladena gymnocephali* sp. nov. (Digenea: Haploporidae) from the bald glassy perchlet *Ambassis gymnocephalus* in Kerala, India

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Abstract

The life cycle of a new haploporid digenean, *Hapladena gymnocephali* sp. nov., infecting the bald glassy perchlet *Ambassis gymnocephalus* from the Chaliyar and Kadalundi rivers of Kozhikode district, Kerala, India, is elucidated. The new species is described in detail, its systematic position discussed and compared with related species, *H. acanthuri* Siddiqi & Cable, 1960 and *H. spinosa* Manter & Pritchard, 1961. *Hapladena gymnocephali* is distinctly different from the two latter species in the shape and size of body, nature of testis, extent of vitelline field and excretory bladder. *Hapladena gymnocephali* has a typical haploporid, two-host life cycle: the gymnocephalous, bi-ocellate, distome cercariae released by the snail *Gabbia travancorica* are ingested by the fish and develop directly into adults without undergoing a metacercarial stage of development. This is the first report of the genus *Hapladena* from India, and also the first report of the life cycle of the genus.

Introduction

Linton (1910) established the genus *Hapladena* with *H. varia* infecting some acanthurid fishes in Florida as the type. Yamaguti (1971) considered *Deredena* Linton, 1910 and *Hairana* Nagaty, 1948 as junior synonyms of *Hapladena* and Overstreet & Curran (2005) agreed with this arrangement. In addition to the type, Yamaguti (1971) listed nine species, *H. ovalis* (Linton, 1910) Manter, 1947; *H. leptotelea* Manter, 1947; *H. sohali* (Nagaty, 1948); *H. magna* (Nagaty, 1948); *H. megatyphlon* Perez Vigueraz, 1957; *H. acanthuri* Siddiqi & Cable, 1960; *H. spinosa* Manter & Pritchard, 1961; *H. tanyorchis* Manter & Pritchard, 1961; *H. nasonis* Yamaguti, 1970, under the genus. Since then, Machida & Uchida (1990) synonymized *H. nasonis* with *H. tanyorchis*, and Nahhas & Carlson (1994) treated *H. megatyphlon* as a synonym of *H. leptotelea*. Thus, at present there are eight valid species under the genus *Hapladena*. As far as is known, the life cycle of the genus *Hapladena* has not previously been established. The only available information is the description of a species of cercaria, *Cercaria caribbea* LII infecting the snail, *Zebrina browniana* in Curcao, The Netherlands Antilles, and its metacercaria which encysts in the open, by Cable (1962), who considered the cercaria and metacercaria as the probable life history stages of *H. varia*, a common parasite of surgeon fishes in Curcao.

While examining the bald glassy perchlet *Ambassis gymnocephalus* from Chaliyar and Kadalundi rivers in Kozhikode district, specimens of a species of *Hapladena* were found in the intestine. Further studies proved that it is a new species and is named *Hapladena gymnocephali* sp. nov. The gymnocephalous, bi-ocellate cercariae of *H. gymnocephali* were recovered from the snail, *Gabbia travancorica*, from Kadalundi and Eranjipalam in Kozhikode district during the months of September–December. Cercariae ingested by the host fish, developed into adult worms in the intestine, without undergoing a metacercarial stage of development.

The recovery of *H. gymnocephali* forms the first report of the genus in India; this is also the first report of the life cycle of the genus *Hapladena*.

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Materials and methods

Ambassis gymnocephalus from the Chaliyar and Kadalundi rivers in Kozhikode district were examined for digenetic trematodes, which were studied using a phase-contrast microscope with or without vital staining. Those used for permanent preparations were fixed in 10% formalin under slight cover glass pressure and stained in alum carmine, following the procedure outlined by Cantwell (1981).

Specimens of the freshwater snail *Gabbia travancorica* were collected from Kadalundi and Eranjipalam during September–December, 2004. Snails were screened in the laboratory for cercariae, which on emergence were supravitally stained with neutral red and studied alive. Genital primordia were observed in lacto acetic carmine-stained cercariae. Measurements were made on heat-killed cercariae. A few infected snails were later crushed and examined for intra-molluscan stages of development.

In order to establish experimental infections in fish, fingerlings of *A. gymnocephalus*, collected from freshwater streams, where *Hapladena* infections do not occur, were kept in beakers containing cercariae and observed for their feeding activity. Fish, which were observed actively ingesting the cercariae, were maintained in aquarium tanks containing river water and sacrificed at intervals to study the course of development of the adult. Eggs released by naturally infected adults were maintained in filtered river water for examination of miracidial development. Newly emerged miracidia were studied under a phase-contrast microscope for structural details.

Descriptions of larvae and adult stages are based on measurements of a minimum of 10 specimens fixed in 10% formalin. Measurements are presented in micrometres; the range is followed by mean values in parentheses. Figures were drawn with the aid of a camera lucida, and details added freehand from observations made on live specimens.

Results

Egg

Eggs (fig. 1a) yellow, reniform, operculate; 52–56 × 36–39 (54 × 38).

Miracidium

Miracidia (fig. 1b) hatched after 5 days of incubation; ovoid, with single eye-spot and many germinal cells; 57–60 × 37–39 (58 × 38).

Redia

Rediae (fig. 1c) develop in the hepatopancreas of *Gabbia travancorica*. Body elongate, saccular, light-brown, filled with a few fully developed cercariae, numerous developing cercariae and germinal balls at different stages of development; 462–669 × 100–154 (524 × 129).

Cercaria

Natural infections were found in 20 of 1370 (1.4%) *Gabbia travancorica* collected from Kadalundi and Eranjipalam in Kozhikode district during a 4-month period, from September to December 2004. Cercariae (fig. 1d and e) emerged diurnally and exhibited positive phototaxis. Freshly emerged cercariae swim actively with lashing movements of their tails. After a few minutes of exposure to water, the tails of cercariae start to swell and elongate forming a prominent, white, opaque structure. At rest the cercaria remains suspended in the water column with the tail directed upward and bent at the base, directing the body also upward.

Description

Gymnocephalous, bi-ocellate, distome cercaria. Body spinose, pyriform, heavily pigmented, 204–290 (249) long, 102–138 (114) wide. Tail narrow, short, translucent in freshly emerged cercariae; 180–210 × 70–87 (194 × 76). Within a few minutes, the tail becomes elongated and expanded into a white, opaque, irregularly wrinkled structure, measuring 495–624 × 109–140 (579 × 124).

Oral sucker sub-terminal, round, 34–41 (39) in diameter. Ventral sucker round, equatorial, 33–41 (37). Prepharynx 9–29 (20) long; pharynx globular, 19–23 (20) in diameter. Oesophagus 71–95 (81) long, bifurcates postero-lateral to ventral sucker. Caeca 34–59 (49) long, extend to near posterior quarter of body. Eye-spots prominent, on either side of pharynx; 9–13 × 9-15 (10 × 11). Distance between eye-spots 23–34 (31). Testis rudimentary, transversely oval, composed of a single mass of cells; 18–33 × 29–42 (24 × 34). Ovary also rudimentary sub-spherical, pre-testicular, 12–14 × 19–27 (13 × 22). Rudimentary hermaphroditic sac ovoid, antero-lateral to ventral sucker, 31–46 × 12–15 (38 × 13).

Excretory bladder I-shaped, 31–34 (32) long; main collecting ducts ascend from antero-lateral walls of bladder, extend to the region of oral sucker, then run backwards to the level of the caecal bifurcation and bifurcate to form anterior and posterior collecting ducts. Excretory pore median at posterior end of body. Flame cells numerous, pattern not determined.

Adult

Development of flukes was observed in experimentally infected *Ambassis gymnocephalus*. Uninfected fish were exposed to cercariae that emerged from *Gabbia travancorica*. Cercariae, actively ingested by the fish, enter its stomach. Cercariae with fragmented tails were found in the stomach 1 h post-feeding. After 24 h, cercarial bodies, which increased slightly, were recovered from the intestine of the fish. These were structurally similar to the cercariae. On the second day, developing adults (fig. 1f and g) were 288–320 × 110–152 (301 × 131), the oral sucker was 39–49 (44), and the ventral sucker was 34–39 (36) in diameter. Eye-spots had started to disintegrate. On the fourth day post-feeding, immature adults had grown to 330–366 × 171–184 (348 × 178), the oral sucker was 42–64 (52) and the ventral sucker was 39–52 (47) in diameter. Eye-spots were lost, and the hermaphroditic sac had increased in size, 52–72 × 49–66 (60 × 57). On the sixth day, the hermaphroditic sac was 56–81 × 49–74 (76 × 64), testis 36–44 × 52–59 (39 × 55), ovary 16–19 × 20–24 (17 × 22), and the body did not show much

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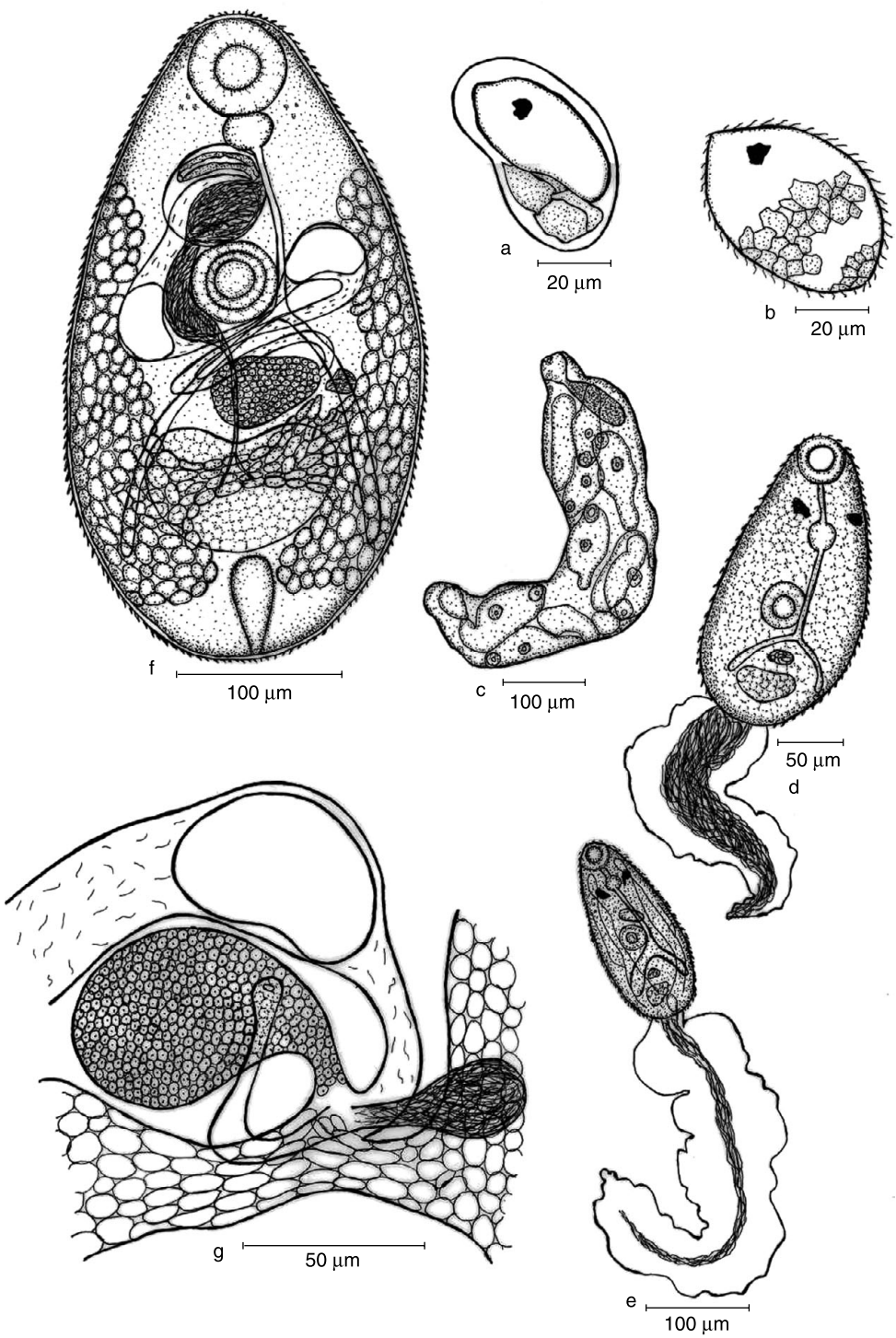

Fig. 1. Life cycle stages of *Hapladena gymnocephali* sp. nov. (a) egg; (b) miracidium; (c) redia; (d) freshly emerged cercaria; (e) cercaria with tail expanded and elongated; (f) adult; (g) ootype complex.

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increase in size. Eggs developed in 7-day-old adults, measuring 346–388 × 178–189 (363 × 182) with an oral sucker 49–62 (55), ventral sucker 49–59 (51), testis 42–52 × 63–69 (47 × 66), ovary 19–23 × 25–29 (20 × 26), and hermaphroditic sac 69–99 × 52–98 (84 × 89).

Natural infections with adult flukes were found in 171 of 249 (68.67%) *Ambassis gymnocephalus* collected from Chaliyar and Kadalundi rivers. The intensity of infection varied from 1 to 15. Naturally infected flukes were identical with those recovered from experimentally infected fish.

Description

Body spinose, pyriform, unpigmented, 361–547 × 178–303 (414 × 236). Body spines 3–8 (5) long. Eye-spot pigment scattered in the region postero-lateral to oral sucker. Oral sucker round, sub-terminal, 51–77 (65) in diameter. Ventral sucker round, muscular, 48–69 (55) in diameter. Sucker ratio 1: 0.77–0.90 (1: 0.85). Fore-body 102–214 (134) long, 31–39% (35%) of body length. Prepharynx 3–15 (8) long; pharynx sub-globular, 23–37 × 27–49 (34 × 38). Oral sucker/pharynx width ratio, 1: 0.5–0.6 (1: 0.57). Oesophagus 62–192 (126) long, and bifurcates postero-laterally to the ventral sucker. Caeca 69–184 (96) long, and terminate blindly 56–136 (85) from posterior end of body.

Testis single, reniform, 36–105 × 99–148 (69 × 112), 21–69 (42) posterior to ventral sucker. Post-testicular field 46–102 (75) long, 13–22% (17%) of body length. Seminal vesicle bipartite; distal portion enclosed within the hermaphroditic sac; internal seminal vesicle sub-globular, 29–74 × 26–62 (41 × 39) and external seminal vesicle 52–99 × 42–52 (72 × 48) in size.

Ovary ovoid, pre-testicular, 21–49 × 39–102 (36 × 71), 3–34 (21) posterior to ventral sucker. Seminal receptacle ovoid, sinistral to ovary, 27–33 × 36–39 (37). Laurer's canal well developed, opens near anterior margin of ovary. Ootype complex postero-lateral to ovary. Uterus extends between ovary and hermaphroditic sac, containing 1–6 eggs and numerous sperm. Hermaphroditic sac anterior to ventral sucker, enclosed metraterm, hermaphroditic duct and internal seminal vesicle 36–138 × 52–115 (65 × 77). Hermaphroditic duct tubular, muscular and eversible. Genital pore median, 12–79 (59) anterior to ventral sucker. Vitelline follicles large, extending from the middle level of the hermaphroditic sac to the post-testicular region, where they are not confluent. Vitelline ducts run transversely at the mid-testicular region to meet the ootype.

Excretory bladder I-shaped, 64–95 (77) long, extends to posterior end of testis, where it receives two collecting tubes. Two pairs of lymph vessels extend along the antero-lateral margins of the body.

Taxonomic summary

Genus: *Hapladena* Linton, 1910.
Species: *Hapladena gymnocephali* sp. nov.
Type host: *Ambassis gymnocephalus* Lacepede, Ambassidae.
Site: Intestine.
Molluscan host: *Gabbia travancorica* (Benson); Bithyniidae.
Type locality: India, Kerala, Kozhikode district, Chaliyar and Kadalundi rivers.
Holotype: Deposited in the Department of Zoology, University of Calicut, Kerala, India. No. Z./Par./Dig.-2004-1a
Paratypes: Z./ Par./ Dig.-2004-1b-c.
Date of collection: 12 June 2004.
Etymology: Named after the species name of the type host.

Discussion

The genus *Hapladena* Linton, 1910 belongs to the subfamily Megasoleninae Manter, 1935 of the family Haploporidae Nicoll, 1914. Of the eight valid species, the present form is comparable to *H. acanthuri* Siddiqi and Cable, 1960 and *H. spinosa* Manter and Pritchard, 1961 in having sucker ratios and egg size which are similar, and in having a median genital pore. A comparison of characters of the three species, presented in table 1, shows that both *H. acanthuri* and *H. spinosa* are distinctly different from the present form in having an elongated body, longer than broad testis, a vitelline field which extends posterior to the ventral sucker and a longer excretory bladder which reaches up to the level of ovary. *Hapladena acanthuri* differs further in having a longer hermaphroditic sac which extends slightly posterior to the ventral sucker, and *H. spinosa* differs in the nature of Laurer's canal and internal seminal vesicle and in the presence of a sphincter around the genital pore. Based on the differences of the present fluke with its closely related forms, it would appear reasonable to treat the present form as a new species of *Hapladena*. It is designated *H. gymnocephali* sp. nov. after the specific name of the type host, *Ambassis gymnocephalus*.

The only available information on the life cycle of the genus *Hapladena* is the description of *Cercaria caribbea* LII, a large gymnocephalous, bi-ocellate cercaria belonging to the family Haploporidae, infecting the snail *Zebrina browniana* in Curacao, The Netherlands Antilles, and its metacercaria, which encysts in the open (Cable, 1962). Based on the morphology and ecology of the larval stages, Cable considered them to be the life history stages of *Hapladena varia*, a common parasite of surgeon fishes in that region. No experimental attempts were made to substantiate this contention. The cercaria of *H. gymnocephali* is different from other known gymnocephalous, bi-ocellate, distome cercariae in its dimensions, the development of the testis, ovary and other reproductive structures, and in the absence of a cyst-forming ability. However, it needs comparison with *C. caribbea* LII, which is supposed to be the larval stage of *Hapladena varia*. The cercaria of *H. gymnocephali* is distinctly different from *C. caribbea* LII in the nature of the tail and in having differentiated testis, ovary and other reproductive structures. Further, it never encysts in the open to form a metacercarial stage of development.

As far as is known, the life cycles of only ten haploporid species are known: *Pseudohapladena pearsoni* (Martin, 1973) Overstreet & Curran, 2005 by Martin

Table 1. Comparison of characters of *Hapladena gymnocephali* sp. nov. with related species; measurements in micrometres.

Character	*Hapladena acanthuri* (Siddiqi & Cable, 1960)	*Hapladena spinosa* (Manter & Pritchard, 1961)	*Hapladena gymnocephali* sp. nov.
Body	Elongate, spines at anterior half only	Elongate,spinose	Pyriform, spinose
	2963–4620 × 785–1254	2111–4288 × 549–734	314–547 × 178–303
Sucker ratio	1:0.92	1:0.79–0.95	1:0.77–0.90
Testis	Entire, elongate-oval, post-equatorial	Elongate, with lateral swelling at the origin of vas deferens	Reniform
	587–752 × 244–415	335–637 × 214–395	36–105 × 99–148
Hermaphroditic sac	Elongated, extending a short distance posterior to ventral sucker	Elongated, almost entirely dorsal to ventral sucker	Sub-spherical, just anterior to ventral sucker
		201–503 × 144–169	36–138 × 52–115
Genital pore	Anterior to ventral sucker	47–112 anterior to ventral sucker surrounded by well-developed sphincter	12–79 (53) anterior to ventral sucker
Vitelline follicles	Extend from the level of ventral sucker to posterior end	Extend from near posterior edge of ventral sucker to tip of caeca	Extend from the middle level of hermaphroditic sac to post-testicular region
Eggs	54–57 × 36–41	Yellow, 53–67 × 35–50	Yellow, reniform, 52–56 × 36–39 (54 × 38)
Host	*Acanthurus caeruleus*	*Acanthurus sandvicensis*	*Ambassis gymnocephalus*

(1973); *Saccocoelium obesum* Looss, 1902, *S. tensum* Looss, 1902 and *Haploporus benedeni* (Stossich, 1887) Looss, 1902 by Fares & Maillard (1974); *Carassotrema koreanum* Tang & Lin, 1979 and *C. wui* Tang & Lin, 1979 by Tang & Lin (1979); *Saccocoelioides carolae* Lunaschi, 1984 by Martorelli (1988); *S. tarpazensis* Diaz & Gonzalez, 1990 by Diaz & Gonzalez (1990) and *Pseudohapladena martini* (Madhavi, 1979) Overstreet & Curran, 2005 and *Carassotrema bengalense* Rekharani & Madhavi, 1985 by Shameem & Madhavi (1991). For all these species, the cercaria is a gymnocephalous, bi-ocellate and distome type, infecting snails belonging to the superfamily Rissooidea and encysting on any available substratum or surface film of water. Definitive hosts are fish and infection is through ingestion of metacercariae. *Hapladena gymnocephali* sp. nov. also possesses a two-host life cycle, but the actively swimming, and structurally different cercariae emerging from the snail, *Gabbia travancorica,* are ingested by *Ambassis gymnocephalus* and develop into adult flukes in its intestine. Cercariae are positively phototactic, and on exposure to water their tails swell and elongate to become prominent, white, opaque structures. This may be an adaptive behaviour shown by the cercaria to attract the definitive host, which is a surface-feeding fish. This is an example of a parasite drawing the attention of the definitive host toward its infective stage for successful transmission. The direct pathway exhibited by *H. gymnocephali* for completion of its life cycle has survival value and is adaptive, as the cercaria is the infective stage developing directly into the adult in the definitive host.

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