The life cycle of *Gyliauchen volubilis* Nagaty, 1956 (Digenea: Gyliauchenidae) from the Red Sea

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Abstract

Although nothing is known about gyliauchenid life cycles, molecular phylogenetic studies have placed the Gyliauchenidae Fukui, 1929 close to the Lepocreadiidae Odhner, 1905. The gyliauchenid *Gyliauchen volubilis* Nagaty, 1956 was found in the intestine of its type-host, *Siganus rivulatus*, a siganid fish permanently resident in a lagoon within the mangrove swamps on the Egyptian coast of the Gulf of Aqaba. Larval forms of this trematode (mother sporocysts, rediae and cercariae) were found in the gonads and digestive gland of *Clypeomorus clypeomorus* (Gastropoda: Cerithiidae), a common snail in the same lagoon. So, this life cycle of *G. volubilis* was elucidated under natural conditions: eggs are directly ingested by the snail; mother sporocysts and rediae reach their maturity 3–6 and 11–13 weeks post-infection; rediae contain 23–29 developing cercariae; fully developed cercariae are gymnocephalus, without penetration glands, emerge from the snail during the night 16–18 weeks post-infection and rapidly encyst on aquatic vegetation (no second intermediate host); encysted metacercariae are not progenetic; 4-day-old metacercariae encysted on filamentous algae fed to *S. rivulatus* developed into fully mature worms 6–8 weeks post-infection. The cycle was completed in about 26 weeks and followed one of the three known patterns of lepocreadiid life cycles, and except for the gymnocephalus cercariae, the other larval stages are very similar to those of lepocreadiids. Generally, the life cycle of *G. volubilis* implicitly supports the phylogenetic relationship of Gyliauchenidae and Lepocreadiidae inferred from molecular phylogenetic studies.

Introduction

The Gyliauchenidae Fukui, 1929 is a small family within the superfamily Lepocreadioidea Odhner, 1905 (see Bray, 2005). Members of this family constitute a group of intestinal trematodes, which are almost entirely found in certain herbivorous teleost fishes (e.g. siganids and acanthurids) in the Indo-West Pacific. *Gyliauchen* Nicoll, 1915, the type-genus of the Gyliauchenidae, was described by Nicoll (1915). Between 1916 and 2004, the most important studies on the family were those of Ozaki (1933, 1934, 1936, 1937), Yamaguti (1934, 1942), Caballero & Bravo-Hollis (1953), Manter & Pritchard (1962), Shalaby & Hassanine (1997), Hall & Chambers (1999) and Hall & Cribb (2004). In these studies, ten gyliauchenid genera have been erected: *Telotrema* Ozaki, 1933, *Petalocotyle* Ozaki, 1934, *Paragyliauchen* Yamaguti, 1934, *Flagellotrema* Ozaki, 1936, *Apharyngogyliauchen* Yamaguti, 1942, *Ichthyotrema* Caballero & Bravo-Hollis, 1953, *Leptobulbus* Manter & Pritchard, 1962, *Progyliauchen* Shalaby & Hassanine, 1997, *Affecauda* Hall & Chambers, 1999 and *Ptychogyliauchen* Hall & Cribb, 2004.

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Paggi & Orecchia (1963) proposed *Robphildollfusium* as a new genus for *Distomum fractum* Rudolphi, 1819 within a new monotypic family, the Robphildollfusiidae Paggi & Orecchia, 1963. In a comprehensive revision of the Gyliauchenidae, Hall & Cribb (2005) reduced the Robphildollfusiidae with its monotypic genus to the rank of a subfamily within the Gyliauchenidae. They also proposed *Hadrobolbus* as a new gyliauchenid genus for three *Flagellotrema* species in which the oesophageal bulb is distinctly large. Accordingly, 13 genera (comprising about 34 species) are currently recognized as valid within the Gyliauchenidae (see Hall & Cribb, 2005). In these genera, the oral sucker is absent and functionally replaced by the pharynx, the ventral sucker is either at or close to the posterior end of the body (except in *Petalocotyle* and *Robphildollfusium*), and the oesophagus is surrounded by dense gland-cells and ends in an oesophageal bulb (except in *Apharyngogyliauchen*); in most species, the oesophagus is very long (longer than the body when straightened), highly convoluted and forms many coils within the fore body. This peculiar morphology and some other characteristics of gyliauchenids appear to partly overlap those of the paramphistomoid family Paramphistomidae Fischoeder, 1901 and have led to speculation about their phylogenetic relationships; some (e.g. Cable & Hunninen, 1942; Cable, 1956; Yamaguti, 1971) regarded them as close to the Paramphistomidae, but others (e.g. Ozaki, 1937; Pearson, 1992) as close to the lepocreadioid family Lepocreadiidae Odhner, 1905. These disputed relationships were tested with molecular data (Barker *et al.*, 1993; Blair & Barker, 1993), which gave very strong support for regarding the Gyliauchenidae as close to the Lepocreadiidae. This was supported by the molecular analyses of Cribb *et al.* (2001), Littlewood *et al.* (2001), Olson *et al.* (2003) and Bray *et al.* (2009).

To date, nothing is known about the gyliauchenid life cycles (see Cribb *et al.*, 1994, 2003; Hall & Cribb, 2005; Bray *et al.*, 2009) and this remains a conspicuous gap in our knowledge of this family. The rabbitfish *Siganus rivulatus* Forsskål, 1775 (Teleostei, Siganidae) is common in the Red Sea and is parasitized by the gyliauchenid trematode *Gyliauchen volubilis* Nagaty, 1956 (see Nagaty, 1956); this parasite appears endemic to the Red Sea. In the present study, the authors take the opportunity to describe the life cycle of this gyliauchenid under natural conditions.

Materials and methods

Some lagoons are scattered within the mangrove thickets found on the Egyptian coast of the Gulf of Aqaba (between 28°7′N and 28°18′N). Lagoons are permanently water filled, even if sometimes at low tides they are completely separated from the sea. In one of these lagoons (*c.* 40 m in diameter and 0.7–1.8 m in depth), only four fish species and six molluscan species (four gastropods and two bivalves) are permanently resident. Of these fishes, the siganid fish *S. rivulatus* was the only one parasitized by the intestinal trematode *G. volubilis* Nagaty, 1956. Larval forms of this trematode (mother sporocysts, rediae and cercariae) were found only in the gonads and digestive gland of the cerithiid

gastropod *Clypeomorus clypeomorus* Jousseaume, 1888; other fishes and molluscs were completely free of trematodes. To prevent fishes from escaping from the lagoon, a plastic net with narrow mesh size was used as a barrier at the lagoon mouth; the lagoon can therefore be considered as a natural aquarium. Fish identifications were based on Randall (1983) and their names follow Froese & Pauly (2004/2009). Mollusc identifications were based on Sharabati (1984). Standard parasitological techniques were used to examine the alimentary canal of the fish and the different organs of the molluscs. Trematodes were removed from their host fishes or molluscs under a stereomicroscope and observed live under a compound microscope; the examination of larval trematodes was facilitated by the use of neutral red as a vital stain, since the stain accumulated rapidly in their bodies.

Examination of numerous specimens of *C. clypeomorus* from a large wild bed inhabiting the coast of Tur-Sinai (180 km south of the mangrove thickets) revealed that they were not naturally infected with any larval trematode. Therefore, 800 specimens from this bed were marked and transferred alive in January 2009 to the lagoon to allow their infection with *G. volubilis* to be followed from its beginning; 50 specimens were dissected and examined weekly. Similarly, and to estimate the approximate time taken by the parasite to reach maturity in its definitive host, some uninfected specimens of *S. rivulatus* were transferred to the lagoon and some were kept alive in aquaria during the study. To detect egg hatching, numerous eggs were taken from fully gravid specimens of *G. volubilis*, concentrated in filtered seawater, placed in embryo cups and observed for more than 4 weeks at 20–25°C. Also, attempts were made to obtain miracidia from the eggs under coverglass pressure, exposure to light and by changing osmotic conditions. To determine whether the cercariae were released from the snails or not, some snails were placed singly in small glass bowls filled with filtered seawater and observed; the addition of a drop of neutral red to the bowls was helpful. The behaviour of cercariae was observed in a Petri dish under the microscope. Measurements made under coverglass pressure vary with the amount of water in the preparation, and usually increase as the water evaporates. More accurate drawings were obtained by killing the larval stages of the parasite, especially the cercariae, in whirling hot water before fixation in hot 5% formalin without pressure. This procedure gives specimens of uniform size and shape. Some mature worms were fixed in alcohol–formalin–acetic acid (AFA) under a very slight coverslip pressure and preserved in 75% ethyl alcohol. Whole mounts were stained in alum carmine, cleared in terpineol and mounted in Canada balsam. Measurements are quoted as the range, with the mean in parentheses, and are given in micrometres, except where indicated. Drawings were prepared with the aid of a drawing tube.

During 2010, this work was partly repeated and confirmed at the mangrove thickets near Rabigh (between 22°49′N and 22°54′N) on the western Red Sea coast of Saudi Arabia, which are similar to those on the Egyptian coast of the Gulf of Aqaba, and where the fish *S. rivulatus* is also parasitized by *G. volubilis* and the larval forms of this trematode are also found in the gastropod *C. clypeomorus*.

Results

Gyliauchen volubilis Nagaty, 1956

Final host. *Siganus rivulatus* Forsskål (Siganidae); site: intestine.

Molluscan host. *Clypeomorus clypeomorus* Jousseaume (Gastropoda: Cerithiidae); site: gonads and digestive gland.

Locality. Mangrove swamps on the Egyptian coast of the Gulf of Aqaba and mangrove swamps near Rabigh on the western Red Sea coast of Saudi Arabia.

Other material examined. The holotype specimen (Reg. no.TRSF.1956.8) in the Parasitology Department, Faculty of Medicine, Cairo, Egypt.

Eggs. Eggs oval, thin-walled, non-operculate, 56–74 × 32–48 (56 × 40). Numerous eggs were taken from fully gravid specimens of *G. volubilis*, concentrated in filtered seawater, placed in embryo cups and observed for more than 4 weeks at 20–25°C, but no hatching was observed. Attempts to obtain miracidia from the eggs under coverglass pressure, exposure to light or by changing osmotic conditions were unsuccessful. As they do not hatch in seawater, infection must be accomplished by the ingestion of eggs and the emergence of miracidia in the digestive tract of *C. clypeomorus*. This was confirmed, when eggs of the same shape and size were observed in the digestive tracts of many infected individuals of this snail. Furthermore, eggshells were observed in their faeces. Thus, there is probably no free-swimming miracidium in the life cycle of *G. volubilis*.

Mother sporocyst (fig. 1A and B). Young mother sporocyst (1–2 weeks after infection) (based on 30 specimens) (fig. 1A): body elongate, tapered at one end, thin-walled, opaque white, 496–757 × 86–140 (627 × 113). Germinal balls aggregated in an elongated cluster at the middle of the body; regions toward the two ends appeared to be transparent.

Fully developed mother sporocyst (3–6 weeks after infection) (based on 30 specimens) (fig. 1B): body elongate, tapered at both ends, thin-walled, opaque white, 2136–2802 × 275–357 (2469 × 316). Few germinal balls and redial embryos remained near the middle of the body; embryos appear to move in opposite directions through the body as they grow into rediae (usually 20–26/sporocyst), which are usually at different stages of development; largest 265 × 89, smallest 65 × 28. Lateral birth-pore observed at 542–846 (694) from the anterior end. The walls of the sporocyst are capable of contraction and distension, but no movement was observed.

Redia (fig. 1C–E). Young redia (7–9 weeks after infection) (based on 30 specimens) (fig. 1C): very mobile. Body elongate, cylindrical, with muscular wall, 207–310 (259) in length, 60–93 (77) in maximum width at its middle. Mouth antero-terminal. Pharynx small, muscular, globular, 10–12 × 12–14 (11 × 13), connected directly to small saccular caecum extending obliquely in anterior fifth of body. Numerous germinal balls and some developing cercarial embryos (10–14/redia) scattered throughout body.

Moderate-sized redia (9–11 weeks after infection) (based on 30 specimens) (fig. 1D): slightly mobile. Body vermiform, 2093–2950 (2522) in length, 340–435 (388) in maximum width at its middle and characterized by

presence of distinct lateral projection at 658–940 (799) from anterior extremity, in the place where the birth pore is situated in fully developed redia. Mouth antero-terminal. Pharynx small, muscular, globular, 66–78 × 74–88 (72 × 81), connected directly to relatively large saccular caecum extending posteriorly to near lateral projection and filled with white droplets which are probably ingested from snail tissue. Few germinal balls and cercarial embryos and some differentiated cercariae (14–19/redia) scattered throughout body.

Fully developed redia (11–13 weeks after infection) (based on 30 specimens) (fig. 1E): immobile. Body vermiform, slightly curved posteriorly, 2970–3570 (3270) in length, 425–540 (482) in maximum width at its middle. Birth pore 1030–1240 (1135) from anterior end and easily seen during emergence of cercariae. Mouth antero-terminal. Pharynx globular, 75–87 × 96–117 (81 × 107), connected directly to large sub-triangular caecum extending posteriorly to level of birth pore and filled with white droplets and amorphous yellow material which are probably ingested from snail tissue. Germinal balls and cercarial embryos completely absent. Differentiated cercariae 23–29 in number, at different stages of development, usually crowded together in anterior half of body.

Cercaria (fig. 1F). After expulsion from the redia, cercariae remain for about 2 weeks within the gonad of the snail before their emergence, which occurs in night.

Fully developed cercaria (emerges from snail 16–18 weeks after infection) (based on 30 specimens): gymnocephalus cercaria. Body pyriform, 198–255 (227) in length, 118–133 (126) in maximum width near its posterior end. Tegument unarmed. Tail simple, long, slender, cylindrical, 2.5–2.7 (2.6) times as long as body, covered by a syncytial tegument overlying muscle layer (capable of extension and contraction), with numerous large vesicular cells arranged longitudinally along its central core, and ends with short invagination (adhesive organ) by which cercariae attach to substrates; invagination contains glandular cells with fine granular materials; the secretion of some sticky substances from these cells may be responsible for adhesion of cercariae to substrates. Oral sucker absent. Ventral sucker spherical, moderately large, 40–50 (45) in diameter, near posterior extremity; hind body very short. Mouth subterminal, surrounded directly by muscular pharynx measuring 24–30 × 19–23 (27 × 21). Oesophagus long, convoluted, with a single loop, surrounded with hardly visible small glandular cells along most of its length. Oesophageal bulb round, 15–21 (18) in diameter. Intestinal caeca simple, relatively wide, extending into middle third of body. Cystogenous gland cells relatively large, ovoid, scattered throughout body except in region occupied by ventral sucker and excretory vesicle; these are negative to neutral red. Penetration glands absent. Testes two, round, symmetrical, dorsal to ventral sucker, subequal, 13–17 (15) in diameter. Anlagen of ovary sinistrally submedian, immediately anterior to ventral sucker. Genital ducts not visible. Excretory vesicle claviform, short, lined by granular cells, extends anteriorly close to ventral sucker and lacks any communication with tail; main excretory tubules unite with antero-lateral margins of excretory vesicle and extend anteriorly to level of intestinal bifurcation. Flame-cell

formula $2[(4+4+4)+(4+4+4)] = 48$. Excretory pore dorso-subterminal.

Metacercaria (fig. 1G). The formation of the metacercaria was observed in a Petri dish filled with seawater and containing small pieces of filamentous algae and other aquatic vegetation on its bottom. The period of the free-swimming cercaria in seawater is very brief. After emergence from the snail, the cercaria appeared to be at rest for only a few seconds and suddenly became very active; it swam through the water using its long tail by
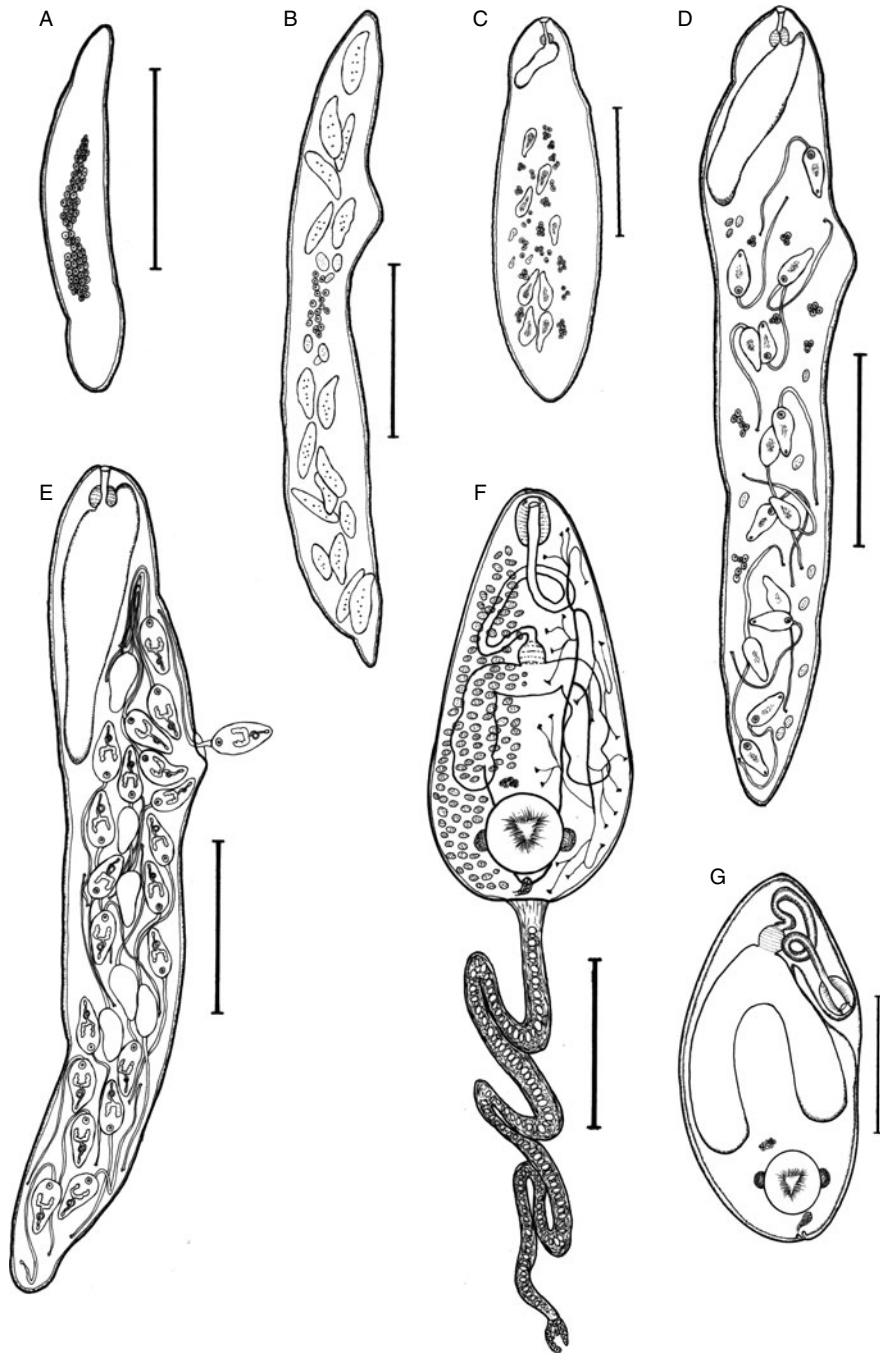

Fig. 1. Stages in the life cycle of *Gyliauchen volubilis* Nagaty, 1956 from the cerithiid gastropod *Clypeomorus clypeomorus*, Red Sea: (A) young mother sporocyst, (B) fully developed mother sporocyst; (C) young redia; (D) moderate-sized redia; (E) fully developed redia; (F) fully developed cercaria; (G) metacercaria. Scale bars: (A) 350 μm; (C, F, G) 100 μm; (B, D, E) 700 μm.

which it propelled itself with a vigorous lashing movement. After a few seconds, the cercaria became less active and swam down to attach to the substratum using its adhesive organ (at the distal end of the tail). At this point, it began to sway with a series of revolving movements which resulted in the detachment of the tail and triggered the emission of secretions from the cystogenous glands. This colourless transparent material forms a flexible membranous cyst around the metacercaria; some of the cyst material is anchored to the substratum. This is probably similar to what happens in nature, since the cercaria has no penetration glands to penetrate a second intermediate host. The metacercariae remained alive for about 1 week and no noticeable progenesis was observed during this period. Generally, it is similar to the cercaria in all characteristics but differs in having a very slightly larger body, wider intestinal caeca and a very small postero-dorsal papilla-like protuberance (excretory papilla). Because the lagoon bottom is very muddy, metacercaria were not seen on the aquatic vegetation, since it is usually covered by a muddy layer. Four-day-old metacercariae encysted on filamentous algae fed to uninfected *S. rivulatus* developed into fully gravid worms 6−8 weeks post-infection. Thus, the complete life cycle of *G. volubilis* extends for about 26 weeks (about 6 months).

Adult (fig. 2). From the intestine of *S. rivulatus* (based on 30 fully gravid-specimens): body fleshy, robust, tapered anteriorly, nearly pyriform, 2361−3775 (3068) in length, 769−1153 (961) in maximum width at its middle, with small postero-dorsal papilla-like protuberance. Tegument unspined. Oral sucker absent. Ventral sucker well developed, spherical, 320−480 (400) in diameter, close to posterior extremity; hind body very short. Pharynx elongate, 202−309 × 136−206 (256 × 171). Oesophagus very long, wide, longer than body length (if straightened), 2965−4377 × 75−118 (3671 × 97), highly coiled, with 2−3 loops, surrounded by glandular cells along most of its length. Oesophageal bulb round, 155−248 (202) in diameter. Intestinal bifurcation at middle of body; caeca simple, wide, extending into middle third of body. Two testes, oval, symmetrical, dorsal to ventral sucker, sub-equal, 321−555 × 220−395 (438 × 308). Cirrus sac short, claviform, mainly intercaecal, 221−352 (287) in length, 112−182 (147) wide at its base, contains tubular seminal vesicle (partly external to cirrus sac), well-developed prostatic complex and relatively short ejaculatory duct; prostatic cells extend outside cirrus sac to cover external part of seminal vesicle. Genital pore median, directly posterior to intestinal bifurcation. Ovary spherical, dextro-submedian between right testis and cirrus sac, 111−175 (243) in diameter. Seminal receptacle oval, submedian, opposite to ovary. Mehlis's gland well developed. Fertilization chamber located between ovary and ootype. Laurer's canal opens on dorsal surface at level of ventral sucker. Uterus relatively short, winds between ovary, left testis and left caecum. Eggs thin-shelled, yellowish, 58−71 × 40−47 (65 × 44). Vitelline follicles relatively small, irregular, numerous, extend in lateral fields between testes and mid-oesophageal level, confluent in region of intestinal bifurcation. Transverse vitelline collecting ducts arising from vitelline follicles on each side of body open into small spherical vitelline
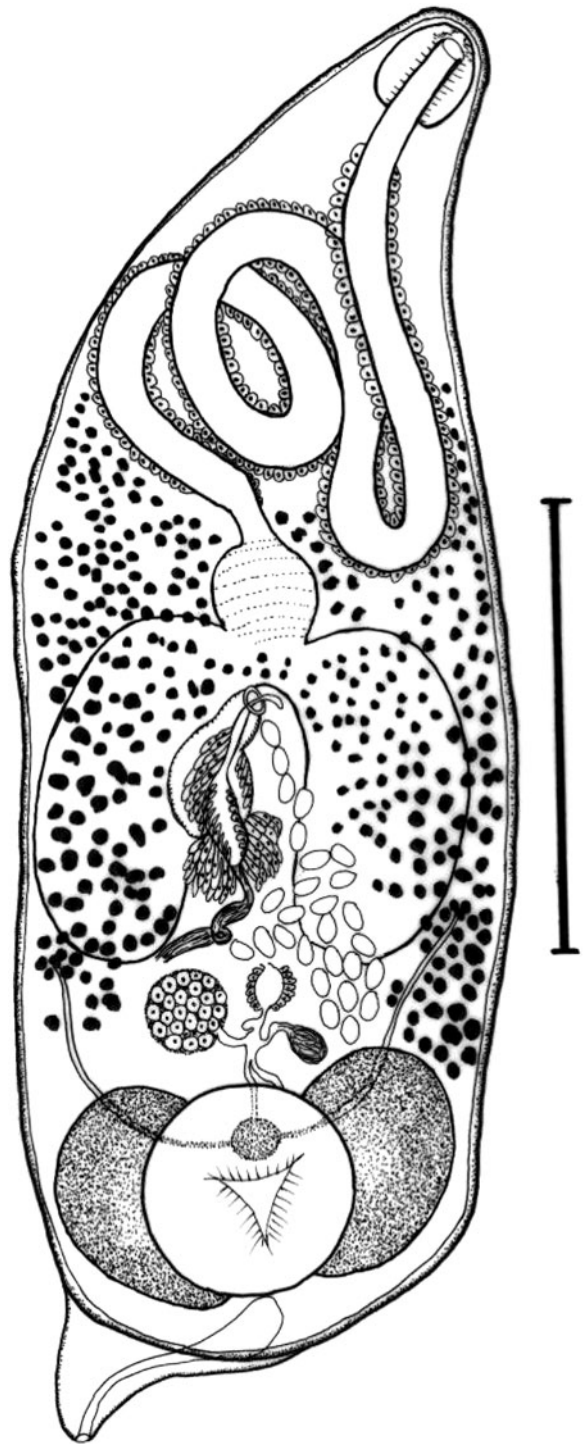

Fig. 2. *Gyliauchen volubilis* Nagaty, 1956 (fully gravid specimen, ventro-lateral view) from *Siganus rivulatus*, Red Sea. Scale bar = 1000 μm.

reservoir dorsal to anterior margin of ventral sucker. Excretory vesicle claviform, extends anteriorly to reach ventral sucker; excretory pore postero-terminal, at tip of papilla-like protuberance (excretory papilla).

Discussion

Although nothing is known about gyliauchenid life cycles (see Cribb *et al.*, 1994, 2003; Hall & Cribb, 2005; Bray *et al.*, 2009), molecular phylogenetic studies have given very strong support to regarding the Gyliauchenidae Fukui, 1929 as close to the Lepocreadiidae Odhner, 1905 (see Barker *et al.*, 1993; Blair & Barker, 1993; Cribb *et al.*, 2001; Littlewood *et al.*, 2001; Olson *et al.*, 2003; Bray *et al.*, 2009). However, these studies predicted that the molluscan hosts in the gyliauchenid life cycles are likely to be gastropods, and the concentration of gyliauchenids in herbivorous fishes suggests that the life cycle might incorporate a cercaria that encysts in the open as a metacercaria on aquatic vegetation, waiting there to be eaten by the fish definitive host (Hall & Cribb, 2005).

The Lepocreadiidae is a large family comprising about 74 genera overwhelmingly parasitic in teleost fishes (see Bray, 2005). The life cycles of some species belonging to only about ten of these genera have been described (see Palombi, 1937; Macfarlane, 1951; Bartoli & Prevot, 1967; Stunkard, 1969, 1972; Lengy & Shchory, 1970; Køie, 1975; Stunkard, 1980a, b; Watson, 1984; Hassanine, 2006). Thus, the life cycles of most lepocreadiid genera are still unknown. The known lepocreadiid life cycles include three distinct patterns, each clearly adapted to the environmental conditions and the trophic patterns of their definitive hosts (see Bray *et al.*, 2009). In one common pattern, eggs hatch in water, free-swimming miracidia penetrate a gastropod as a first intermediate host, ophthalmotrichocercous cercariae (with the eyespots and tails bearing setae) produced in rediae, emerge from the gastropod and penetrate an invertebrate (or occasionally small vertebrate) as a second intermediate host, and wait there to be eaten by the definitive host (carnivorous fish). In the second pattern (restricted to deep-sea forms adapted to lack of light), eggs hatch in water, free-swimming miracidia penetrate a gastropod as a first intermediate host, cercariae with no eyespots and with stumpy tail lacking setae produced in rediae, emerge from the gastropod and usually penetrate an annelid as a second intermediate host, and wait there to be eaten by the definitive host (deep-sea fish). In the third pattern, eggs are ingested by a bivalve (no free-swimming miracidium), trichocercous cercariae produced in rediae, emerge from the bivalve and encyst in the open as a metacercaria on a potential food source of the definitive host, and the metacercaria waits there to be eaten by the definitive host (herbivorous or benthic grazing fish). Our study revealed that the life cycle of *G. volubilis* clearly follows the latter pattern, but involves a gastropod rather than a bivalve as the molluscan host.

Lepocreadiid mother sporocysts have been described in four studies (see Stunkard, 1969; Lengy & Shchory, 1970; Watson, 1984; Hassanine, 2006). In other studies, a mother sporocyst was not observed, but it was assumed that if it did exist, it would disappear soon after a single brood of rediae had been liberated (e.g. Macfarlane, 1951). The mother sporocyst of *G. volubilis* is closely similar to other lepocreadiid mother sporocysts and is readily found in the gonads of *C. clypeomorus*. All lepocreadiid rediae reported in the literature were only briefly described.

Characteristics of the life-history stages, especially the cercarial morphology, are of great importance in studying the phylogenetic relationships between the groups and subgroups of digenean trematodes (Pearson, 1972; Odening, 1974; Bozhkov, 1982; Brooks *et al.*, 1985; Gibson, 1987; Cribb *et al.*, 2003). The known lepocreadiid cercariae are almost entirely trichocercous, i.e. their tails bear setae (rarely with stumpy tail lacking setae), whereas the cercariae of *G. volubilis* are distinctly gymnocephalus. These cercariae greatly resemble *Cercaria queenslandae* VIII Cannon, 1978 from the cerithiid gastropod *Cerithium moniliferum*, off Heron Island, Great Barrier Reef. This cercaria was assumed to be of the family Gyliauchenidae by Cannon (1978).

Lepocreadiid metacercariae are progenetic and ovigerous (Macfarlane, 1951), growing for a short period in the cyst (Watson, 1984), or not progenetic (Bartoli & Prevot, 1967). The metacercariae of *G. volubilis* are not progenetic.

Cable & Hunninen (1942) suggested that the peculiar body shape of gyliauchenids may be due to the failure of the hind body to grow during post-cercarial development, with a compensating elongation of the fore body such that the so-called excretory papilla would then correspond to the hind body of other trematodes. Our study does not support this suggestion, since the developing cercaria of *G. volubilis* has a greatly reduced hind body (similar to the fully developed stage) and the excretory papilla begins to appear in the metacercaria; the failure of the hind body to grow may occur during the early development of cecariae within the redia. However, the peculiar gut morphology, i.e. the long convoluted oesophagus surrounded by dense cells, appears during the later development of the cecariae within the redia. Based on the ultrastructure, Jones *et al.* (2000) interpreted the peculiar gut morphology of gyliauchenids to be an adaptation to the predominantly herbivorous diets of the definitive fish host.

The life cycle of *G. volubilis* is similar to the typical life cycle of Paramphistomidae, but in the latter, the eggs hatch in water and the released miracidia penetrate the molluscan host (see Durie, 1956; Cribb *et al.*, 2003). However, the larval stages of *G. volubilis* (except the cercaria) are very similar to those of paramphistomids. Controversially, the adult stages of *G. volubilis* and all other gyliauchenids possess a genital complex and an excretory system that clearly differ in structure from those of paramphistomids, but are clearly similar to those of lepocreadiids.

In the light of the above, the life cycle of *G. volubilis* follows one of the three known patterns of lepocreadiid life cycles and, except for the gymnocephalus cercariae, the characteristics of the other larval stages of this gyliauchenid are closely similar to those of lepocreadiids. Thus, the life cycle of *G. volubilis* implicitly supports a phylogenetic relationship between the Gyliauchenidae and the Lepocreadiidae, as inferred from molecular phylogenetic studies.

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