

DIONCHUS POSTONCOMIRACIDIA (MONOGENEA: DIONCHIDAE) FROM THE SKIN OF BLACKTIP SHARKS, *CARCHARHINUS LIMBATUS* (CARCHARHINIDAE)

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ABSTRACT: Postoncomiracidia of *Dionchus* sp. are described from specimens collected from the skin of 2 blacktip sharks *Carcharhinus limbatus*, captured in the northern Gulf of Mexico. The parasites resemble nonciliated oncomiracidia hatched from eggs laid by *Dionchus* sp. on gills of a cobia *Rachycentron canadum* and adults of *Dionchus remorae* that were collected from gills of a common sharksucker *Echeneis naucrates*, captured in association with a third blacktip shark. The hamuli of the postoncomiracidia were morphologically similar to those of adult *D. remorae*. This is the first report of dionchids from an elasmobranch and from a location other than the gills. These findings support the idea that some dionchid oncomiracidia colonize the skin of sharks or other aquatic vertebrates that sponsor remoras, prior to transferring to other remoras and maturing.

Dionchidae Bychowsky, 1957 (Monogenea) contains 6 known species: *Dionchus agassizi* Goto, 1899, *Dionchus remorae* (MacCallum, 1916), *Dionchus rachycentris* Hargis, 1955, *Dionchus nagibinae* Timofeeva, 1988, *Dionchus johnstoni* Gupta and Sharma, 1989, and *Dionchus rauschi* Gupta and Sharma, 1989. Dionchids have only been reported from a few teleosts, including several members of Carangidae (e.g., see MacCallum, 1916; Radha, 1975; Ramasamy et al., 1985; Gupta and Sharma, 1989) and Echeneididae (e.g., see MacCallum, 1916; Hargis, 1955; Koratha, 1955a; Ktari, 1977; Gupta and Sharma, 1989), and from monotypic Rachycentridae (e.g., see Hargis, 1955; Koratha, 1955a, 1955b; Rohde, 1978). Dionchids tether their eggs firmly to the gill filaments of their hosts (see Ktari, 1977; Kearn, 1986; Whittington, 1990), and it has been suggested (Ktari, 1977) that upon hatching from eggs attached to the gills of a remora, some oncomiracidia might colonize the fish to which the remora was attached. Because these infected sponsor fishes are visited by other remoras, their skin might serve as important platforms from which *Dionchus* infections can spread throughout remora populations. Herein, we provide the first direct evidence of this phenomenon by describing postoncomiracidia of *Dionchus* sp. incidentally collected from the skin of blacktip sharks while studying *Dermophthirius penneri* Benz, 1987 (Monogenea: Microbothriidae).

MATERIALS AND METHODS

Skin samples observed to be infected with *D. penneri* were collected from 2 blacktip sharks, *Carcharhinus limbatus* (Valenciennes, 1839), caught by long-lining in the northern Gulf of Mexico during August 1996 and 1997. Skin samples were fixed in 95% ethanol or 10% neutral buffered formalin and examined microscopically for parasites. A common sharksucker *Echeneis naucrates* L., 1758, that came aboard attached to a third blacktip shark was also fixed in 10% neutral buffered formalin. In the laboratory, eggs and adults of *D. remorae* were collected from the gills of the fixed sharksucker and stored in 10% neutral buffered formalin for later study. Egg bundles that contained live embryos of *Dionchus* sp. were collected from the gills of a freshly killed cobia *Rachycentron canadum* L., 1766, that had been captured during August 1998 in the northern Gulf of Mexico. These egg bundles were brushed to remove fish mucus and debris and were maintained for

hatching at room temperature in a dish of filtered seawater for approximately 1 hr. Dead, partially emerged oncomiracidia were brushed gently from hatched eggs and fixed in 10% neutral buffered formalin. Live oncomiracidia were observed stereoscopically and as wet mounts using phase contrast microscopy. Postoncomiracidia from the shark skin, egg bundles and adults from the sharksucker, and egg bundles and oncomiracidia from the cobia were routinely processed, stained with Semichons carmine or cleared in clove oil, and permanently mounted on glass slides using neutral Canada balsam. Mounted specimens were examined using brightfield, phase-contrast, and Nomarski interference microscopy, and measurements were made using an ocular micrometer. Illustrations were made using a camera lucida or drawing tube.

Several shark skin patches with attached postoncomiracidia and several detached postoncomiracidia of *Dionchus* sp. and eggs and adults of *D. remorae* from the gills of the sharksucker were studied using scanning electron microscopy (SEM). Prior to SEM, these samples were placed in 100% ethanol (2 changes, 1 hr each), immersed in hexamethyldisilazane (15 min), dried using a slight vacuum to remove the hexamethyldisilazane, mounted on metal stubs with 2-sided sticky tape, and sputter coated with gold-palladium.

Monogeneans were identified using information contained in MacCallum (1916), Price (1938), Hargis (1955), Bychowsky (1961), Ktari (1977), Benz (1987), and Whittington (1990). Voucher specimens of postoncomiracidia of *Dionchus* sp. collected from shark skin and oncomiracidia of *Dionchus* sp. hatched from eggs collected from the gills of the cobia have been deposited in the U.S. National Parasite Collection (USNPC) at Beltsville, Maryland (postoncomiracidia USNPC no. 89124; oncomiracidia USNPC no. 89123) and at the H. W. Manter Laboratory (HWML) at Lincoln, Nebraska (postoncomiracidia HWML no. 15057; oncomiracidia HWML no. 15056).

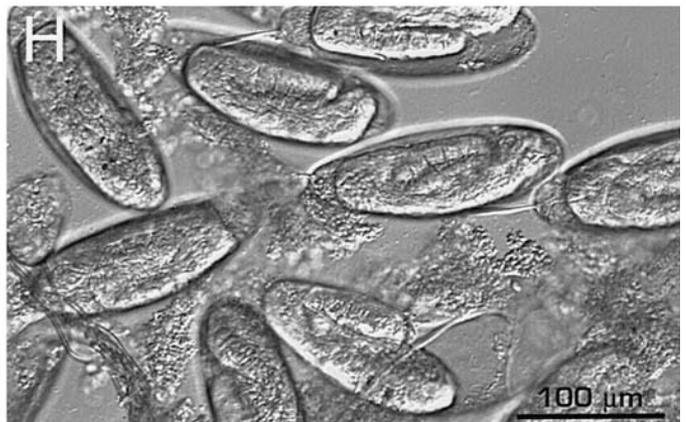
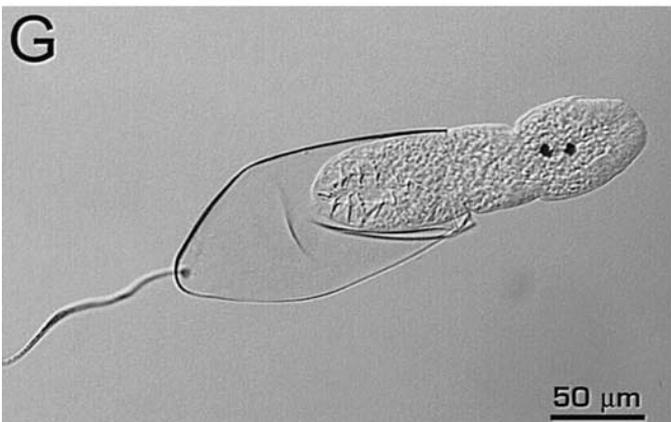
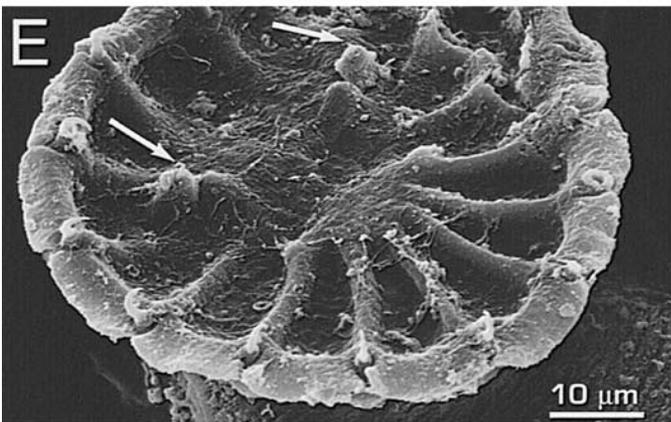
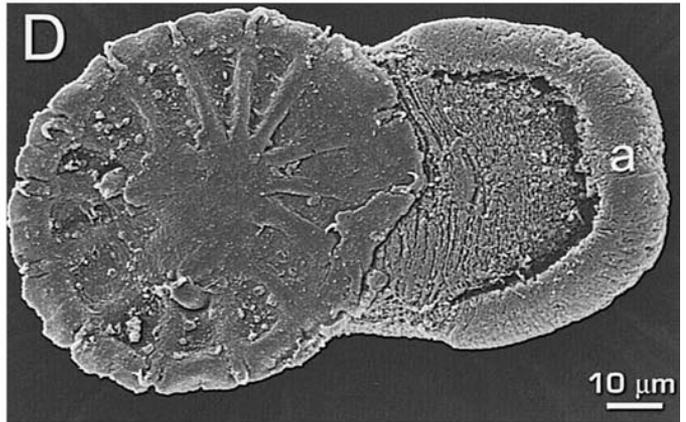
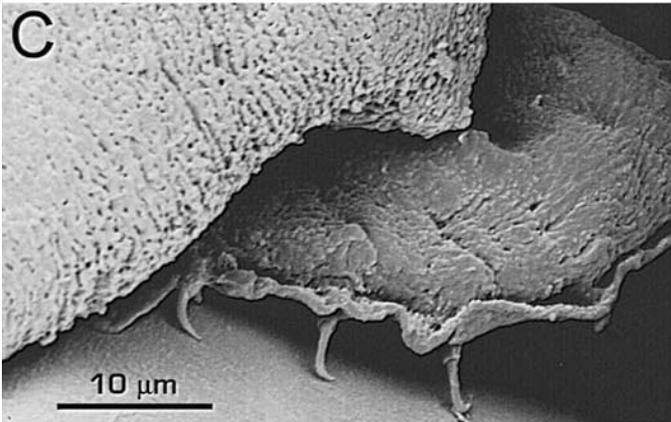
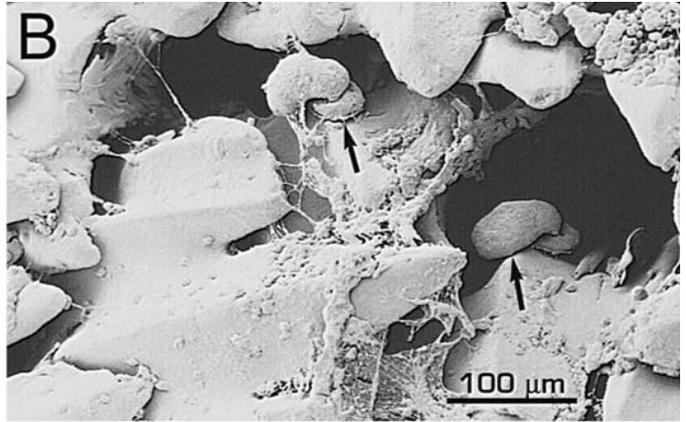
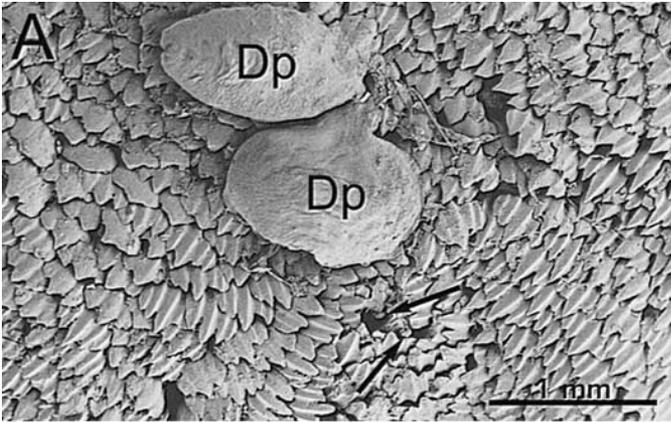
RESULTS

Twenty postoncomiracidia of *Dionchus* sp. and many eggs, juveniles, and adults of *D. penneri* were collected from the examined shark skin patches (Fig. 1A). The haptors of the postoncomiracidia were attached to the crowns of placoid scales (Fig. 1B, C), and in some instances the worms were attached to scales that appeared out of normal alignment and recessed relative to nearby scales (e.g., Fig. 1B). Fixed postoncomiracidia are oval or kidney-bean shaped, 94–203 μm ($n = 18$) long, and 62–89 μm ($n = 16$) wide across the haptor. Their epithelium is nonciliated. They have 2 pairs of dorsal eyespots located in the anterior half of the body (Fig. 2A–C), each consisting of a cluster of brown spherical granules. Postoncomiracidia have a ventral structure resembling an adhesive cephalic gland that is 11–12 μm ($n = 5$) wide that wraps around the anterior of the body and extends posteriorly almost half of the body length to the level of the posterior pair of eyespots (Figs. 1D, 2B, C). The haptor is circular and has 14 marginal haptoral

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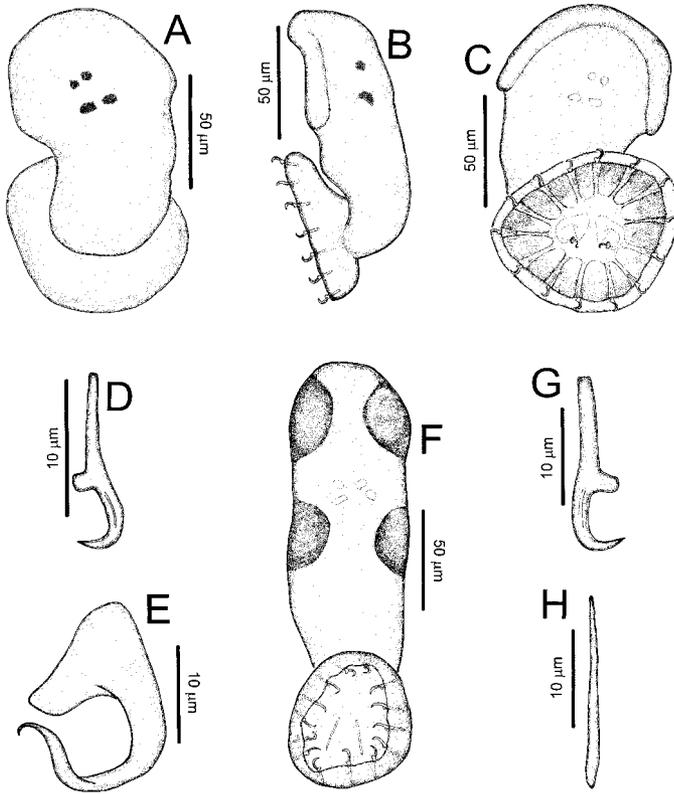


FIGURE 2. Postoncomiracidium of *Dionchus* sp. from skin of a blacktip shark (A–E) and oncomiracidium of *Dionchus* sp. from cobia (F–H). A. Dorsal view. B. Lateral view. C. Ventral view. D. Marginal haptor hooklet. E. Hamulus. F. Ventral view. G. Marginal haptor hooklet. H. Hamulus.

hooklets and 14 radial septa that form 14 loculi that encircle an aseptate central region (Figs. 1E, 2C). Each of the 14 marginal hooklets (Fig. 2D) are 10–12 μm ($n = 7$) long and are located at the junctions of adjacent loculi around the haptor periphery (Figs. 1E, 2C). Haptor hooklets consist of a shaft, guard, and blade (Fig. 2D). The hooklet shaft (Fig. 2D) is 6–7 μm ($n = 5$) long and is rooted in the distal portion of the septum (Fig. 2C). The hooklet guard (Fig. 2D) is located approximately in the middle of the hooklet and is exposed (Fig. 2C). The hooklet blade (Fig. 2D) is exposed (Figs. 1C, 2D), curves approximately 90° relative to the long axis of the shaft, gradually narrows along its length to form a sharp tip, and possesses conspicuous grooves on both lateral surfaces. Two hamuli (Fig. 2E) are located in the center of the haptor (Figs. 1E, 2C). Each hamulus has a relatively long base that is 9–11 μm ($n = 5$)

long that narrows into a corkscrew-like blade that distally forms a slightly recurved sharp tip. Tips of hamuli protrude from the ventral surface of the haptor and are not covered by tissue (Figs. 1E, 2C). General shape and location of hamuli are similar to those of examined adult *D. remorae*.

Eggs of *Dionchus* sp. from gills of the cobia are operculate, lightly tanned, 209–222 μm ($n = 8$) long and 64–69 μm ($n = 10$) wide, and grouped in bundles as described by Whittington (1990). Each egg has a single polar filament (Fig. 1G) that distally joins a loop that functions as a lasso to tether the egg bundle to a gill filament. Just prior to hatching, embryos of *Dionchus* sp. filled much of the eggs, and their eyespots and marginal haptor hooklets were clearly visible through the egg shell (Fig. 1H). These embryos were oriented with their anterior ends nearest the opercula and haptors tucked ventrally (Fig. 1H).

Oncomiracidia emerged from egg bundles collected from gills of the cobia within 1 hr of observation. Hatching was roughly simultaneous and was not induced by any known stimulus. Newly emerged oncomiracidia crawled rapidly about by stretching and contracting their bodies in a looping, inchworm-like fashion. Oncomiracidia are 159–174 μm ($n = 9$) long and 55–62 μm ($n = 9$) wide. Their epithelium is nonciliated and they have 2 pairs of eyespots located in the anterior half of the body (Fig. 2F) that are each composed of brown spherical granules. A well defined adhesive cephalic gland is not evident. However, oncomiracidia have lateral pads (Fig. 2F) located in the anterior (1 pair) and middle (1 pair) of the body that are glandular in appearance and are each 36–38 μm ($n = 4$) long and 17–21 μm ($n = 4$) wide. The haptor is aseptate, circular, cuplike, and 46–52 μm ($n = 7$) in diameter. Oncomiracidia have 14 marginal haptor hooklets (Fig. 2G) that are 18–19 μm ($n = 5$) long and evenly spaced around the haptor periphery (Fig. 2F). Aside from being relatively longer, these hooklets are morphologically similar to those of the postoncomiracidia collected from the shark skin. Two bar-shaped hamuli (Fig. 2H) that are 19 μm ($n = 5$) long are located in the center of the haptor with the anterior tips lying closer together than the posterior tips (Fig. 2F). Hamuli are similar in shape but not in size to those reported by Ktari (1977) for oncomiracidia of *D. agassizi*.

DISCUSSION

Live oncomiracidia of *Dionchus* sp. that we studied were highly mobile. Ktari (1977) suspected that larvae of some species of *Dionchus* are capable of moving from 1 remora to another by colonizing the external surface of fish that sponsor remoras. Nonciliated dionchid larvae would likely colonize sites on the sponsor fish that are most often frequented by rem-

FIGURE 1. Scanning electron micrographs of postoncomiracidia of *Dionchus* sp. from skin of a blacktip shark (A–E) and adult *Dionchus remorae* from gills of a common sharksucker (F), and light micrographs of eggs and oncomiracidia of *Dionchus* sp. from gills of a cobia (G, H). A. Locations (arrows) where 2 postoncomiracidia are attached near 2 much larger adult *Dermophthirius penneri* (Dp) on skin of a blacktip shark. Note the counterclockwise whirled appearance of the placoid scales that possibly indicates scarring associated with *D. penneri* infections. B. Close-up of same 2 postoncomiracidia as in Figure 1A. Note that these 2 worms are attached to the crowns of placoid scales that appear out of normal alignment and recessed relative to nearby scales. C. Haptor of postoncomiracidium attached to a placoid scale. D. Ventral view of postoncomiracidium from skin of a blacktip shark. Adhesive cephalic gland = a. E. Ventral view of haptor of postoncomiracidium from skin of a blacktip shark. Arrows point to exposed tips of hamuli. F. Haptor of adult *D. remorae* from gills of a common sharksucker. Arrows point to exposed tips of hamuli. G. Oncomiracidium emerging from egg. H. Embryonated eggs from gill filament of a cobia.

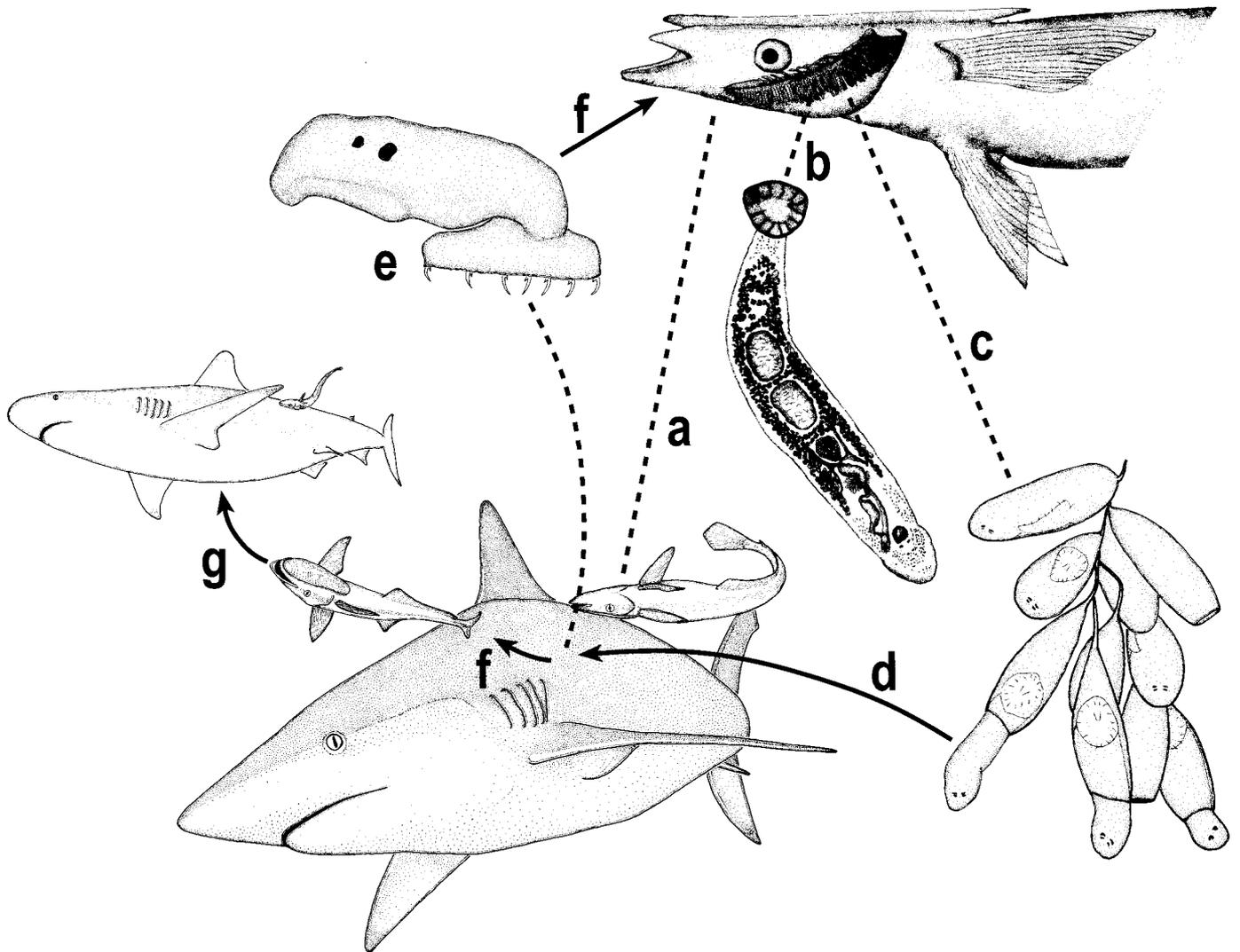


FIGURE 3. Proposed life cycle for some species of *Dionchus*. Infected remora (a) mingles with a sponsor fish. Adult *Dionchus* sp. reside on the gills (b) and permanently tether egg bundles (c) to the gill filaments. Oncomiracidia hatch and at least some crawl from the remora's branchial chamber onto the sponsor fish (d). Postoncomiracidia (e) use sponsor fish as a platform from which they colonize remoras (f). Infections of postoncomiracidia are spread throughout populations of sponsor fish when infected remoras periodically move to other fish (g).

oras, and casual observations of remoras attached to whale sharks *Rhiniodon typus* Smith, 1828, and large mobulids Mobulidae, indicate that remoras have specific locations where they rest on the sponsor and feed on ectoparasites. Many ectoparasites of marine fish are site specific (e.g., see Rohde, 1982; Benz, 1986), and the existence of specific attachment sites may facilitate the routine attraction of remoras to particular body regions. The colonization of remora resting and feeding sites by dionchid larvae could increase the likelihood that resting remoras would become infected by crawling postoncomiracidia or that remoras would become infected while feeding.

Monogenean hatching can involve chemical cues from fish mucus or mechanical cues from host activity (Kearn, 1986). However, we noted that the egg bundles of *Dionchus* sp. from the gills of a cobia hatched in the absence of any apparent stimulus. It is possible that a hatching stimulus may not be necessary for some species of *Dionchus*. Remoras spend considerable time attached

to other fish. This behavior could allow crawling larvae ample opportunity to infect fish that sponsor remoras. Here, it is noteworthy that remoras often ram ventilate when they are attached to moving fish by opening their mouths and opercula to allow water to flow passively over their gills (Muir and Buckley, 1967). We suspect that this behavior also provides crawling dionchid larvae easy passage from and to the gills of some remoras.

Our discovery of postoncomiracidia of *Dionchus* sp. on the skin of 2 blacktip sharks provides the first direct evidence supporting the idea first advanced by Ktari (1977) that dionchid larvae may infect the skin of fish that sponsor remoras. Based on our results and on information contained in the literature, we propose the following life cycle for at least some individuals of some species of *Dionchus* (Fig. 3). Eggs attached to the gill filaments of a remora hatch, and oncomiracidia crawl from the branchial chamber, through the opercular opening, and onto the external body surface of the remora. Some of these larvae crawl

from the body surface of the remora to the body surface of a sponsor fish. Remoras may orally acquire postoncomiracidia from colonized sponsor fish while feeding on other ectoparasites (e.g., see Cressey and Lachner, 1970). These postoncomiracidia attach to the remora's gill arches and then crawl to the gill filaments to feed, mature, copulate, and produce eggs. Alternatively, postoncomiracidia could crawl from the external surface of the sponsor fish onto an attached remora and proceed to its gills either via the mouth or opercula.

The differences we noted in the relative degree of exposure of the marginal hooklets and hamuli of postoncomiracidia of *Dionchus* sp. from the skin of the sharks versus the same structures of adult *D. remorae* from the gills of the common sharksucker could be due to fixation artifacts. They could also represent interspecific or ontogenetic variation, or both. Furthermore, they may also indicate alternate uses of the haptoral sclerites that are associated with the different habitats of young versus adult worms. The marginal haptoral hooklets of postoncomiracidia could facilitate attachment to the hard placoid scales of elasmobranchs via a coordinated grasping action (see Fig. 1C), whereas the larger and more exposed adult hamuli could be used to pierce the relatively soft gill tissues of teleosts. Regarding this matter, it is interesting that Kearn (1978) noted ontogenetic differences between the haptors of juvenile and adult *Horricauda rhinobatidis* Young, 1967 that were possibly associated with a habitat shift.

We believe that the life cycle proposed above provides a reasonable explanation of how a nonciliated dionchid oncomiracidium could colonize a remora. Monogeneans that possess nonciliated oncomiracidia typically infect hosts that reside in or on a substrate, whether this substrate is, for example, sand or, as in the case of remoras, a living shark (e.g., Llewellyn, 1963, 1981; Ktari, 1977). However, it is more difficult to explain how dionchid infections spread among nonphoretic, pelagic hosts (i.e., members of Carangidae and the cobia). It is probable that the tethered egg bundles of dionchids facilitate infection among tightly schooling pelagic fishes such as carangids by not allowing eggs to fall from the gills prior to hatching. However, how the nonciliated oncomiracidium disperses itself within carangid populations remains unknown, and this matter seems even more problematic regarding cobia populations because cobia typically congregate in relatively smaller (see Böhlke and Chaplin, 1993) and looser groups. Fournier and Combes (1979) noted (as reported in Kearn, 1986) that the monogenean *Eupolystoma alluaudi* (Beauchamp, 1913) can produce nonciliated and ciliated oncomiracidia. Nonciliated oncomiracidia are used in the process of autoinfection, whereas ciliated oncomiracidia are used for dispersal throughout host populations and are produced when an infrapopulation becomes overcrowded (see Kearn, 1986). Possibly the life cycles of some dionchids involve both nonciliated and ciliated oncomiracidia. Lastly, it should be noted that cobia are sometimes accompanied by remoras (e.g., see Böhlke and Chaplin, 1993), and it is possible that remoras might become infected with dionchids from 1 cobia and ferry the juvenile worms to other cobia.

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