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Neobenedenia girellae (Monogenea) Infection of Cultured Cobia *Rachycentron canadum* in Taiwan

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ABSTRACT—A benedeniid parasite infecting the body surface of cobia *Rachycentron canadum* cultured in net cages in Taiwan was identified as *Neobenedenia girellae*. This is the first confirmed case of *N. girellae* infection of marine fish from Taiwan. *N. girellae* was not randomly distributed on the host; it concentrated on the dorsal side of the head area (59.7%), especially on the eyes (23.7%), while it was less frequent on the ventral side and not detected on the fins. *N. girellae* caused considerable histological damage to the host through the attachment by the haptor and possibly through feeding activity by the pharynx. In infected eyes, epithelial cells of the cornea were often partially lost, and the collagenous stroma was considerably thickened and edematous, associated with massive inflammatory cell infiltration.

Key words: *Neobenedenia girellae*, *Rachycentron canadum*, pathology, cobia, Taiwan

Culture of cobia *Rachycentron canadum* (Rachycentridae) has been rapidly growing in some Southeast Asian countries and in Japan, but most intensively in Taiwan. Net cage culture of cobia started in Taiwan in 1995 and yielded 4,186 metric tons in 2003 according to the FAO statistics. Because of its short culture history, reports on the diseases of cobia are quite limited, including viral, bacterial and parasitic infections (Chen *et al*., 2001; Chi *et al*., 2003; Lopez *et al*., 2002; Rajan *et al*., 2001). A *Sphaerospora*-like myxozoan in the kidney and monogenean *Neobenedenia* sp. on the body surface have been known as parasitic agents (Chen *et al*., 2001; Lopez *et al*., 2002).

Lopez *et al*. (2002) reported that *Neobenedenia* sp. infection was associated with the ulcer formation of the head of cobia, but gave no direct evidence on the pathogenicity of the monogenean. Recently, we had a chance to investigate *Neobenedenia* infection of cobia cultured in Liu-chiu Hsu Island, Taiwan. This parasitic infection was the cause of the recent mass mortality recorded from October 2002 to January 2003, and farmers had to treat the fish with freshwater dips at one-week intervals during September to December. The present paper deals with the identification of the parasite and description of the observed features of the disease, with some notes on the site preference of the parasite on the fish body surface.

Materials and methods

Cobia *Rachycentron canadum* (0-year-old; body length 12.0–37.0 cm; n = 14) were sampled from net cages in Liu-chiu Hsu Island, Taiwan in February 2003, and benedeniid parasites were collected from the body surface. Fresh parasites were flattened between a slide glass and coverslip, and fixed in AFA (70% ethanol 20 parts, formalin 1 part, acetic acid 1 part). They were stained with aceto-carmine, dehydrated, cleared in xylene and mounted in Canada balsam for morphological observation under a light microscope.

The eyes of most of the fish sampled in February 2003 were rendered opaque by parasites (Fig. 1). Opaque eyes from four fish were fixed in Bouin's solution and processed for histological examination. Intact eyes were similarly sampled from three fish cultured in the same farm in March 2003. Paraffin sections, 5 μm thick, were stained with haematoxylin and

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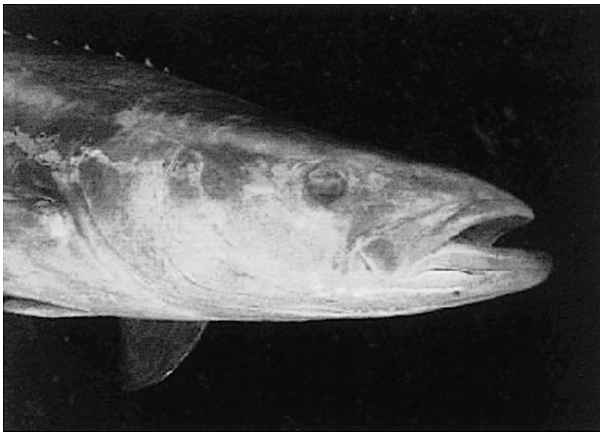

Fig. 1. Cobia *Rachycentron canadum* with opaque eyes.

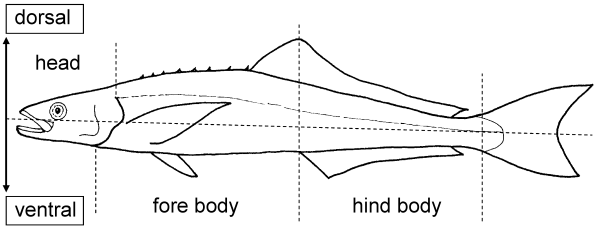

Fig. 2. Body parts (head, fore body or hind body; ventral or dorsal) and fins of cobia *Rachycentron canadum* to record the location of *Neobenedenia girellae*.

eosin. Additionally, some selected sections were stained with Giemsa to examine possible infections with pathogens other than the benedeniids.

To determine parasite distribution on the fish body surface, 0-year-old cobia (body length 16.0–26.0 cm; n = 11) were randomly sampled from the same locality in March 2003. Benedeniid parasites were grossly detected and their location on the body, which was divided into six body parts and fins (Fig. 2), was recorded for each fish.

Wild fish around the net cages were also collected and examined for the benedeniid infection. They were *Dascyllus trimaculatus* (Pomacentridae; 10.2–10.8 cm in body length; n = 3), *Lutjanus erythropterus* (Lutjanidae; 10.0–10.5 cm in body length; n = 2), and *Antennarius* sp. (Antennariidae; 5.8 cm in body length; n = 1).

Voucher specimens of the parasite are deposited at the Meguro Parasitological Museum, Tokyo, M. P. M. Coll. No. 18826.

Results

Measurements and identification of the parasite

Measurements: Body 3.5–5.5 mm in length and 1.7–2.9 mm in maximum width; a pair of anterior attachment organs 0.29–0.47 mm long by 0.27–0.47 mm wide; haptor 0.79–1.20 mm long; anterior hamuli 0.18–0.38 mm long, posterior hamuli 0.12–0.18 mm long, accessory sclerites 0.14–0.28 mm long; a pair of testes 0.34–0.64 mm long by 0.31–0.60 mm wide; ovary 0.19–0.34 mm long by 0.30–0.45 mm wide; vitelline reservoir 0.32–0.51 mm long by 0.08–0.16 mm wide; egg 0.018–0.023 mm long by 0.020–0.023 mm wide.

Identification: The parasite belonged to the subfamily Benedeniinae according to Whittington and Kearn (1993). Briefly, it had an aseptate haptor with three pairs of median sclerites and seven pairs of marginal hooks; anterior attachment organ consisted of disc-

Table 1. Distribution of *Neobenedenia girellae* on the body surface of cobia *Rachycentron canadum*

	dorsal	ventral	total
head	83 (33)*	18	101 (33)*
fore body**	21	13	34
hind body**	3	1	4
fins	0	0	0
total	107	32	139

Figures show the number of *N. girellae* collected from 11 fish.
*: no. of *N. girellae* on the eyes indicated in parenthesis
**: excluding fins

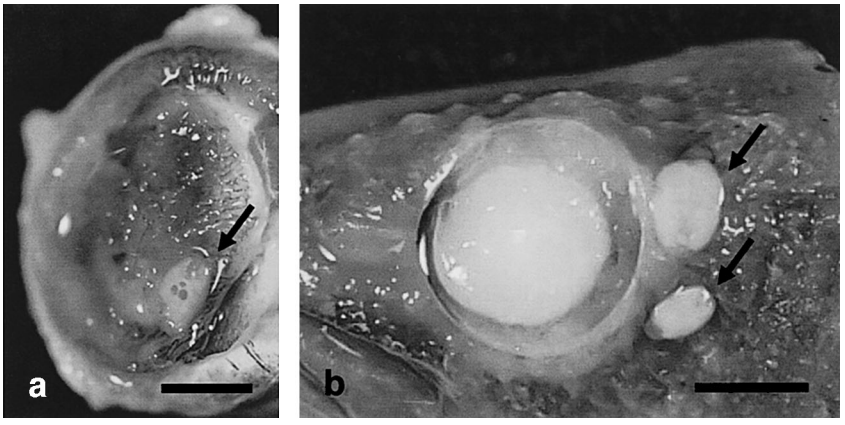

Fig. 3. *Neobenedenia girellae* (arrows) attaching to the eye (a) or to the head part close to the eye (b) of cobia *Rachycentron canadum*. Fixed in Bouin's solution. Scale bar: 3 mm for a; 5 mm for b.

shaped pads; male and female genital apertures opened near the left side of body. Further, the parasite was included in the genus *Neobenedenia*, on the grounds that it lacked vagina and that the accessory gland reservoir (= prostatic reservoir) was contained in the penis sac (Whittington and Horton, 1996). Finally it was identified as *Neobenedenia girellae*, as the above measurements were in well accordance with those of this species described from fishes of Japan (Ogawa *et al*., 1995).

Parasite distribution on the fish body

Table 1 shows the results of 11 cobia sampled in March 2003. All fish were infected and intensity of infection was 12.6 parasites/fish with a range from one to

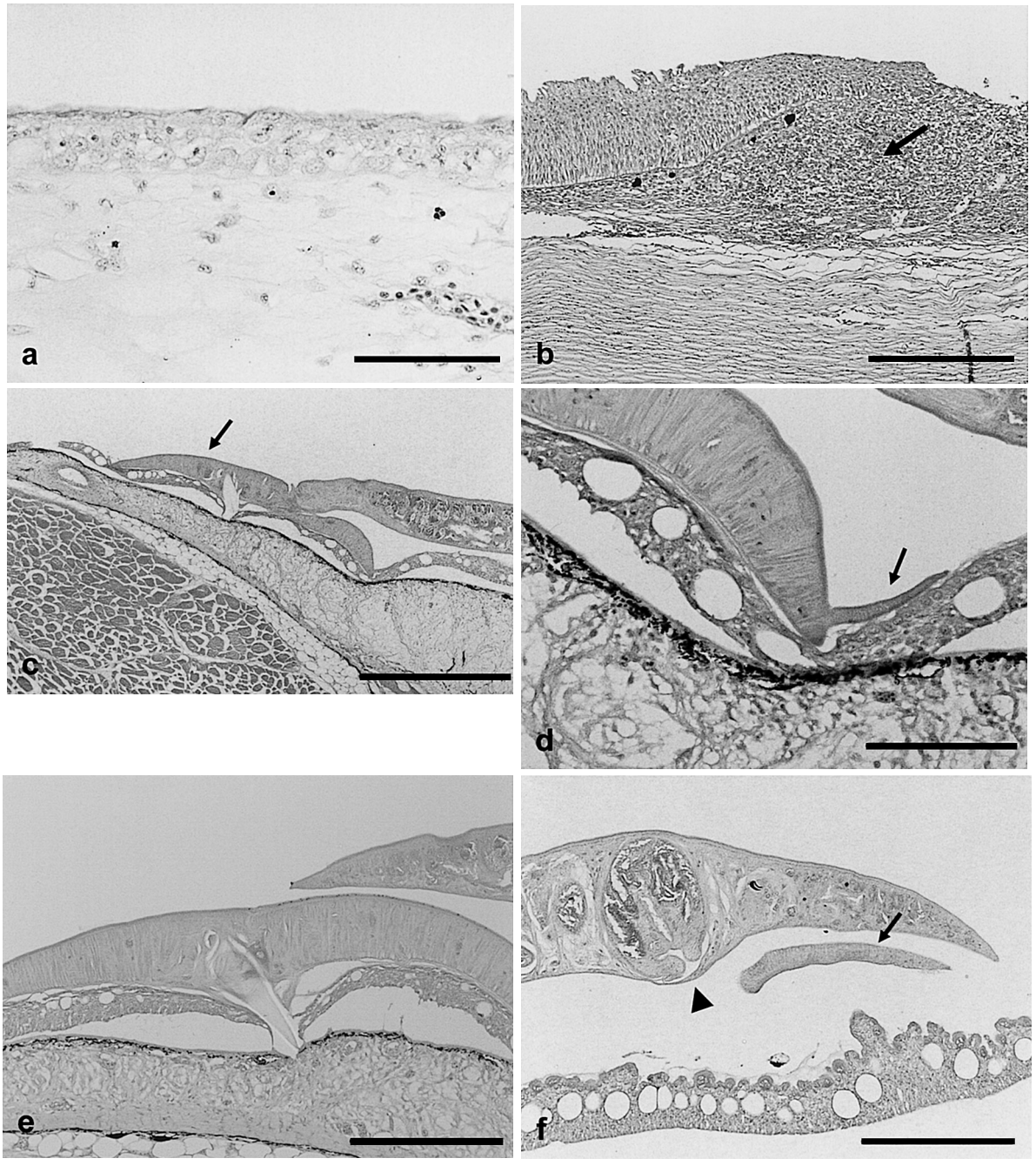

Fig. 4. Histological sections of cobia *Rachycentron canadum* infected with *Neobenedenia girellae*. **a**, cornea of an uninfected fish; **b**, damaged cornea and thickened collagenous stroma, probably caused by the infection; **c**, posterior part of the parasite attaching to the edge of the eye; the haptor is indicated by the arrow; **d**, a higher magnification of **c**; the arrow indicates the haptoral membrane; **e**, accessory sclerite of the anterior hamulus disrupting the epithelium of the host; **f**, anterior part of the parasite near the eye; arrow indicates anterior attachment organ; arrowhead indicates pharynx. Scale bar: 0.1 mm for a and d; 0.2 mm for b, e and f; 0.5 mm for c.

33 parasites/fish. No infection was detected in the three species of wild fish around the net cages: *D. trimaculatus*, *L. ervthropterus* and *Antennarius* sp..

Of a total of 139 parasites collected from cobia, 56 (40.3%) and 83 (59.7%) were found on the right and left sides of the body, respectively. Although more parasites were found on the left side, when examined individually, only one of the 11 fish showed a biased distribution of parasites on the body (eight on the right and 19 on the left body) (binominal test; $p < 0.05$). In this study, results from the right and left side were summed for each of the body areas (Fig. 2; Table 1) from the 11 fish. Since the eight areas were not equal in size, no statistical analysis was made for this apparently biased distribution.

Considerably more parasites were attached to the head region, including the eye (72.7%) (Fig. 3a, b), than the remaining areas (27.3%), and more attached to the dorsal side (77.0%) than to the ventral side of the body (23.0%). This is reflected by a strongly biased distribution on the head part; 83 parasites (82.2%) were collected from the dorsal side of the head including the eyes, compared to 18 from the ventral side (17.8%). Parasites were particularly abundant on the eyes. About one third of parasites collected from the head region concentrated in this relatively small area (33 parasites out of 101 parasites from the head including the eyes). The prevalence of infection on the eye was 91% (10/11) with the highest number recorded from a single eye being six. From head to tail, parasites were most densely distributed in the head region and became less abundant towards the posterior end. No parasite was found on the fins.

Histopathology of the eyes

The cornea of uninfected fish had several layers of squamous epithelial cells almost uniform in size and shape (Fig. 4a), and mucous cells were rarely observed. In fish with parasites on the eyes, the eyes turned opaque, and the corneal epithelial cells were no longer uniform in size or shape, but irregularly thickened and often partially lost (Fig. 4b). Beneath the epithelium, the upper half of the collagenous stroma was considerably thickened and edematous, associated with massive inflammatory cell infiltration (arrow in Fig. 4b). No infection with bacteria or other pathogens was detected in Giemsa-stained sections.

Since parasites were easily detached from the eyes during the histological procedures, only two parasites are shown attaching to the edge of the eye. The parasite attached to the host epithelium by the haptor and a pair of the anterior attachment organs. The haptor (arrow in Fig. 4c) and its surrounding membrane (arrow in Fig. 4d) firmly attached to the epithelium and almost no space was observed between the haptor/membrane and the host tissue. The ventral surface of the haptor was lined with mucus and cell debris. Since these dead cells were more abundant under the haptor than outside the haptor, this suggests the haptor exerts a strong adhesive action. The accessory sclerite of the anterior hamulus disrupted the epithelium at the point of its penetration to the skin (Fig. 4e). The anterior attachment organs (arrow in Fig. 4f) did not always firmly attach to the epithelium underneath. However, the epithelial cells in close contact with the parasite body proper, especially the area near the anterior attachment organs and pharynx (arrowhead in Fig. 4f), were irregularly arranged and deformed, and were no longer uniform in size or shape (Fig. 4f). The surface of the epithelium was covered with mucus, suggesting a strong irritating effect caused by the attachment by the anterior attachment organs and feeding by the pharynx.

Discussion

Two species of monogeneans are known to infect cobia: *Dionchus rachycentris* on the gills (Florida, U. S. A.) (Hargis, 1955) and *Neobenedenia* sp. on the body surface (Taiwan) (Lopez *et al*., 2002). No description was given of *Neobenedenia* sp. by Lopez *et al*. (2002), but from their photo it appears to be the same benedeniid as the one in the present paper. This is the first confirmed case of *Neobenedenia girellae* infection of marine fish from Taiwan.

There are at least two possibilities about the origin of the parasite in the present case, though neither is conclusive. In the same area, there was another farm where amberjack *Seriola dumerili* introduced from mainland China were cultured. Amberjack seedlings, occasionally infected with *N. girellae*, have been exported from mainland China into Japanese waters (Ogawa *et al*., 1995). It would not be surprising if the infection of cultured cobia may have originated from introduced amberjack, as was the case in Japan. Alternatively, it is also possible that the infection was endemic in this area. This is because the monogenean appears to be a parasite in tropical regions (Bondad-Reantaso *et al*., 1995) and shows no host specificity (Ogawa *et al*., 1995). In this hypothesis, wild fish around the net cages may have been the source of infection, although no such infection was confirmed in the present study. This may be due to the small sample size. To establish effective control measures, further investigation will be needed to discover the infection cycle at the culture site.

Bondad-Reantaso *et al*. (1995) showed the high reproductive potential of *N. girellae*. Eggs of this parasite entangled on net meshing will hatch out within four days, and established larvae will mature in about two weeks at 30°C. In this situation, to establish effective control measures may be very difficult, but to determine the infection cycle and evaluate its reproductive potential

is of primary importance.

In Japan, culture nets are frequently changed, because the net meshing provides a good substrate for eggs of *N. girellae*. Freshwater bathing of infected fish for a few minutes is the most commonly practiced method to eradicate another benedeniid monogenean *Benedenia seriolae* for cultured amberjacks *Seriola* spp. The same treatment is applied to *N. girellae*-infected amberjack *S. dumerili* and tiger puffer *Takifugu rubripes*, though it has not been confirmed whether *N. girellae* is as susceptible to freshwater as *B. seriolae* (Kinami *et al*., 2005). Alternatives to a freshwater bath include hydrogen peroxide and praziquantel treatment (Ogawa, 2004), but bathing with hydrogen peroxide should be done very carefully because of its high toxicity to fish at higher temperatures (> 25°C).

N. girellae tended to concentrate on the eyes of cultured cobia. Similarly, in tilapia, *Oreochromis mossambicus*, infected with *N. melleni* (=*N. girellae*; Whittington and Horton, 1996), parasites were found attached to the skin of the anteriodorsal portion of the head and the eyes (Kaneko II *et al*., 1988). Probably, the same mechanisms will have accounted for the apparently biased distribution in the present case too. Another case of biased distribution was reported in rainbow trout *Oncorhynchus mykiss* experimentally infected with the monogenean *Gyrodactylus derjavini* (Buchmann and Bresciani, 1998). The parasite initially attached itself all over the host body, with a preference for the pelvic, pectoral and anal fins, but after six weeks it occurred more densely on the caudal fin and cornea. Interestingly, the low population density of mucous cells in the latter two sites,, especially in the cornea, suggests that mucous cells might play a decisive role for the site selection of *G. derjavini*.

In cobia, mucous cells are sparsely distributed in the cornea. We speculated that the host manages to suppress infection on the skin to some degree by secreting some unidentified substance(s) (probably complement) contained in the mucus. Some of the parasites attached to the skin may become detached, but others escape onto the eyes, resulting in a concentration of infection on the eyes, and possibly leading to blindness of the host. No such clear concentration is known to occur in *N. girellae* infection of tiger puffer cultured in Japan. This might be because the host defense mechanism on the body surface of tiger puffer is not as strong as in cobia.

In cobia, *N. girellae* infection evidently caused considerable histological damage to the host tissue around the eyes (Figs. 3 and 4). As the haptor acts like a strong sucker, its attachment induced mechanical damage to the skin. The host tissue close to the parasite's anterior part was also affected, which suggests that feeding activity of *N. girellae* may also play an important role in lesion formation. Further, marked mucus secretion from the epithelium in contact with the parasite body indicates that this kind of contact is a strong irritant to the host. In *N. melleni* infection of tilapia, Kaneko II *et al*. (1988) reported that the infected skin showed hyperaemia, hemorrhages, sloughing and loss of scales, etc. and that the eyes had corneal opacity, and, in advanced stages, buphthalmos and ulceration of the cornea, leading to blindness. In this study, no other causative agent was observed and all the eyes infected with *N. girellae* had pathological changes including corneal opacity and ulceration, implying that the lesions of the eyes were caused by this parasite.

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