3.2.16 Centrocestiasis (gill trematode disease)

Andrew Mitchell and Andrew Goodwin*

U.S. Department of Agriculture
Agricultural Research Service
H K Dupree-Stuttgart National Aquaculture Research Center
P.O. Box 1050
Stuttgart, AR 72160-1050
870/673-4483

*Aquaculture and Fisheries Center
University of Arkansas at Pine Bluff, Mail Slot 4912
1200 North University Drive, Pine Bluff, Arkansas 71601,
870/575-8137

A. Name of Disease and Etiological Agent.

Centrocestiasis is an infection of the gills of fish by a heterophyid trematode *Centrocestus formosanus* (Nishigori, 1924). Common names for the parasite include "gill trematode" and "mystery fluke."

B. Known Geographical Range and Host Species of the Disease.

1. Geographical Range

The gill trematode has been reported from fish in China, India, Israel, Japan, Malaysia, Mexico, Philippines, Taiwan, Thailand, and Vietnam. In the United States, it has been reported in Florida, Hawaii, Texas and Utah. Cases in the US have all been associated with tropical climates or warm springs.

2. Host Species

The life cycle of *C. formosanus*, includes two intermediate and one definitive or final host.

First intermediate hosts – aquatic mollusks.
Three aquatic snails from the family Thiaridae - *Melanoides tuberculata,* the red-rimmed melania, *Stenomelania newcombi* and *Thiara scabra* - are known to carry developing stages of *C. formosanus* but only *M. tuberculata*, an exotic snail, is now found in the United States in about 15 western, northwestern and southern states. *Melanoides tuberculata* is viviparous and reproduces parthenogenetically. These two factors enhance its ability to reproduce and spread. Young are born live at about 2 mm and can reach a length of 70 to 80 mm although they are more commonly 10 and 40 mm (Figure 1).

Figure 1. Red-rimmed melania (*Melanoides tuberculata*); snail vector of the gill trematode (*Centrocestus formosanus)*. Scale bar major divisions in cm.

Second intermediate hosts – fish and some frogs

The gill trematode is not host specific for freshwater fish, therefore all species should be considered as potential hosts (the parasite is found in the branchial tissue). Frogs, particularly of the genus *Rana*, should also be considered as potential hosts (the trematodes are found in the stomach wall and in the muscle).

Definitive or final hosts – birds and mammals

Aquatic birds in North America known to host the parasite include the black crown night heron *Nycticoras nycticorax* and the green backed heron *Butorides striatus*. Because several aquatic bird genera are reported as final hosts worldwide, almost any fish eating bird should be considered as a possible host. Some fish-eating mammals including rats, cats and dogs, are also reported as natural definitive hosts throughout the world.

C. Epizootiology

Trematodes are released from their cysts in the branchial tissues of fish by the digestive juices in the intestinal tract of their final hosts. The worms released into the lumen of the gut develop into egg producing adults in a few days. Eggs are shed by adult trematodes into the lower intestines of the final host and are defecated into the water. Miracidia, the first larval stages, hatch from the eggs, then enter the snail; reports on the way of entry differ. Some report that the eggs hatch in the water in about two weeks, releasing miracidia that penetrate the snail's foot. Others report that the eggs are eaten by the snails and the miracidia hatch in the snail's intestine and penetrate through the snail intestinal wall. Either way, the miracidia locate in the snail's digestive gland and begin to produce asexually giving rise to cercariae (hundreds of thousands of cercariae can be produced from one miracidium). Cercariae are the second larval stage and infective to fish. They emerge from the snails, are shed in the surrounding water, and search for suitable fish hosts.

The highest shedding rate occurs from snails 20 to 25 mm in length. The average number of cercariae released daily from one red-rimmed melania is more than 1,600 however a release rate of 63,400 cercariae in 24 h from one snail has been observed. Most shedding occurs during daylight. The cercariae of *C. formosanus* survive in waters with temperatures of 15 – 35°C (ideal range being 15 – 25°C). The survival temperature range of the host snail (17-32°C) is within that of the cercaria, therefore, anywhere the snail survives, year round infections of this trematode can occur. The cercariae can survive for 2 to 5 days in water without the host fish but are probably only infective to fish within the first 48 h. The cercaria possesses a straight slender tail, heart-shaped body, two prominent eye spots, and two suckers (an oral and a well-developed ventral sucker) (Figure 2).

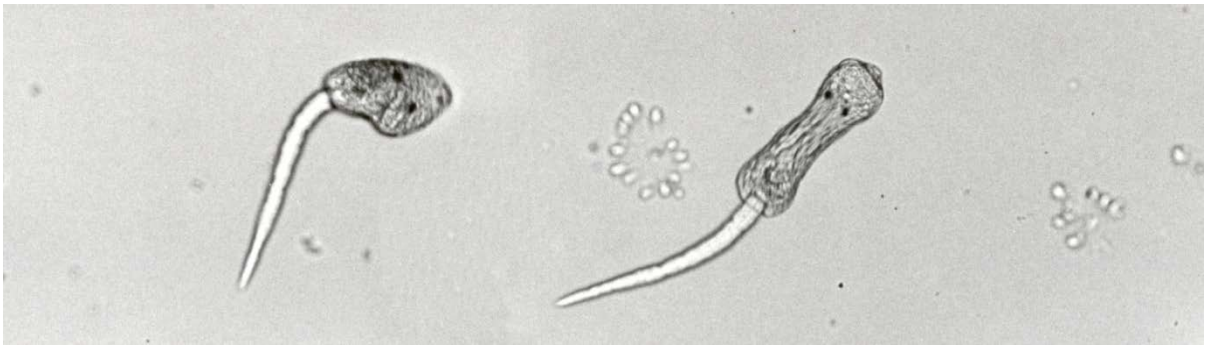

Figure 2. Cercaria of *Centrocestus formosanus*. Note eye spots and straight, slender, non-forked tail. Total length of cercaria (including tail) is about 260 µm.

The cercarial body is 113 to 154 µm long and 76 to 103 µm wide and the tail length is 114 to 155 µm long and 18 to 24 µm wide. The cercariae penetrate the fish host gill tissues and migrate to positions near the gill filament cartilage (Figure 3 video).

Figure 3 Video. Cercaria of *Centrocestus formosanus* orient along the filament cartilage shortly after penetration.

They become metacercariae, surrounded by cysts of parasite origin that have thin inner walls about 2µm thick. As they mature the cysts are further surrounded by a cyst of host origin. Immature metacercariae still have the paired eye spots evident and their cysts are about 100 µm in length (Figure 4 video).

Figure 4 Video. Immature metacercaria of *Centrocestus formosanus* (parasite cyst about 100 µm in length) encysted in the gill filament of a fish. Eye spots are still present.

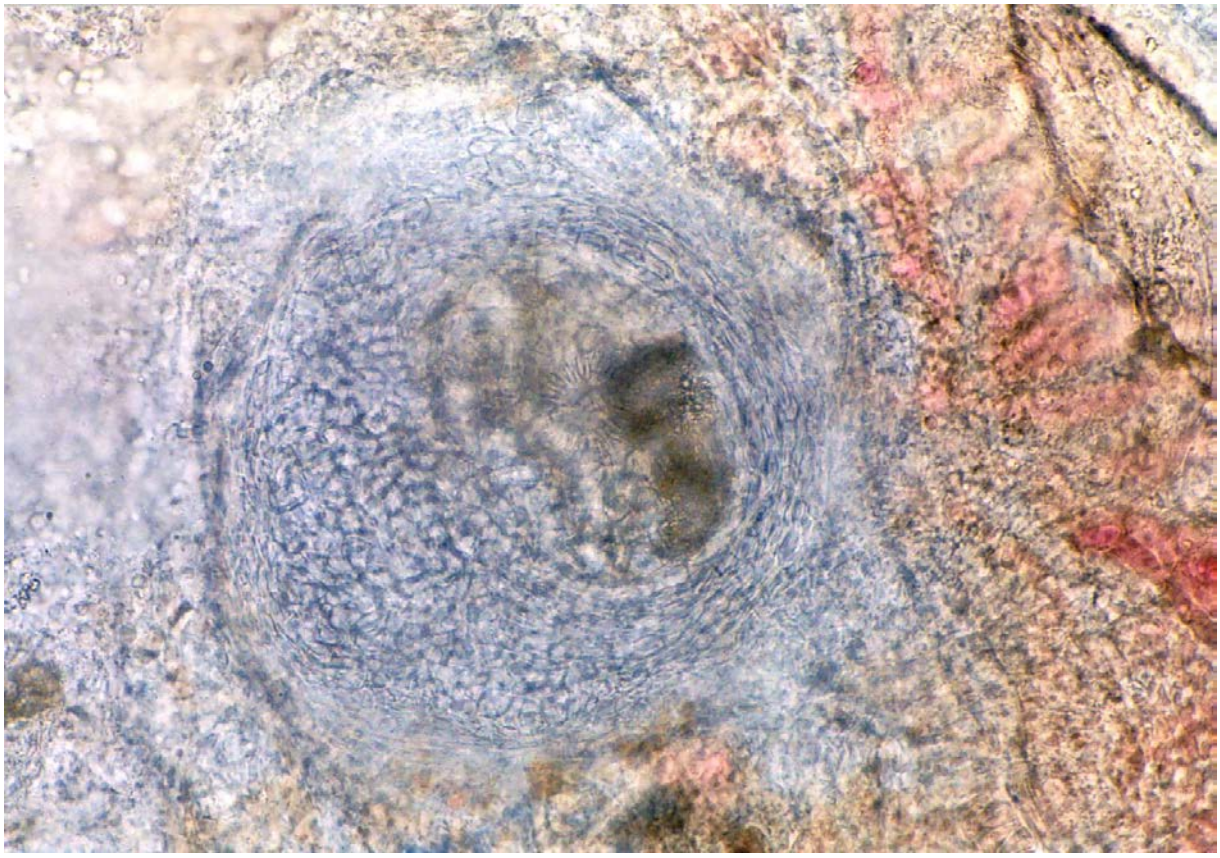

Figure 5. Maturing *Centrocestus formosanus* (parasite cyst about 150 µm in length) encysted in the gills of a fish: a) photo above and b) video showing circumoral spines..

They begin to mature and grow becoming ovoid metacercarial cysts of about 150 µm (107 – 225 µm) in length and of about 110 µm (78 - 159 µm) in width. Maturing metacercariae usually develop two offset rows of circumoral spines (16 spines per row around the oral sucker - Figure 5a, 5b video) and X-shaped excretory vesicles containing dark excretory granules (Figure 6a, 6b video).

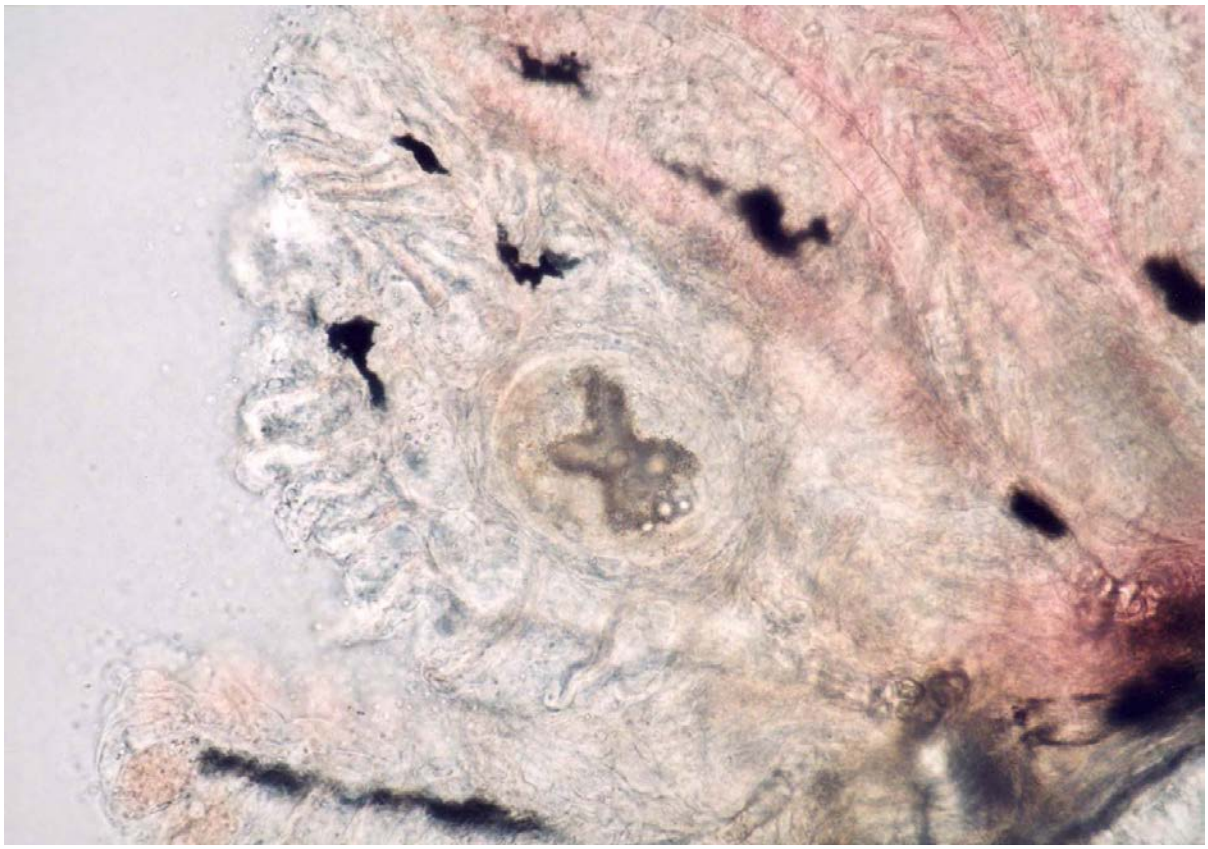

Figure 6. Maturing *Centrocestus formosanus* (parasite cyst about 150 µm in length) encysted in the gills of a fish: a) photo above and b) video showing X-shaped excretory vesicles.

Further development of the metacercaria is delayed until a final host eats the fish. Some metacercariae grow slower, remain smaller, retain eyespots and develop no circumoral spines. Some fish, such as greenthroat darters *Etheostoma leidum* and fountain darters *Etheostoma fonticola*, have host responses that kill the maturing metacercariae and these dead cysts (eye spots remain visible) measure about 95 µm (73 – 114 µm) by 49 µm (36 – 66 µm) (Figure 7).

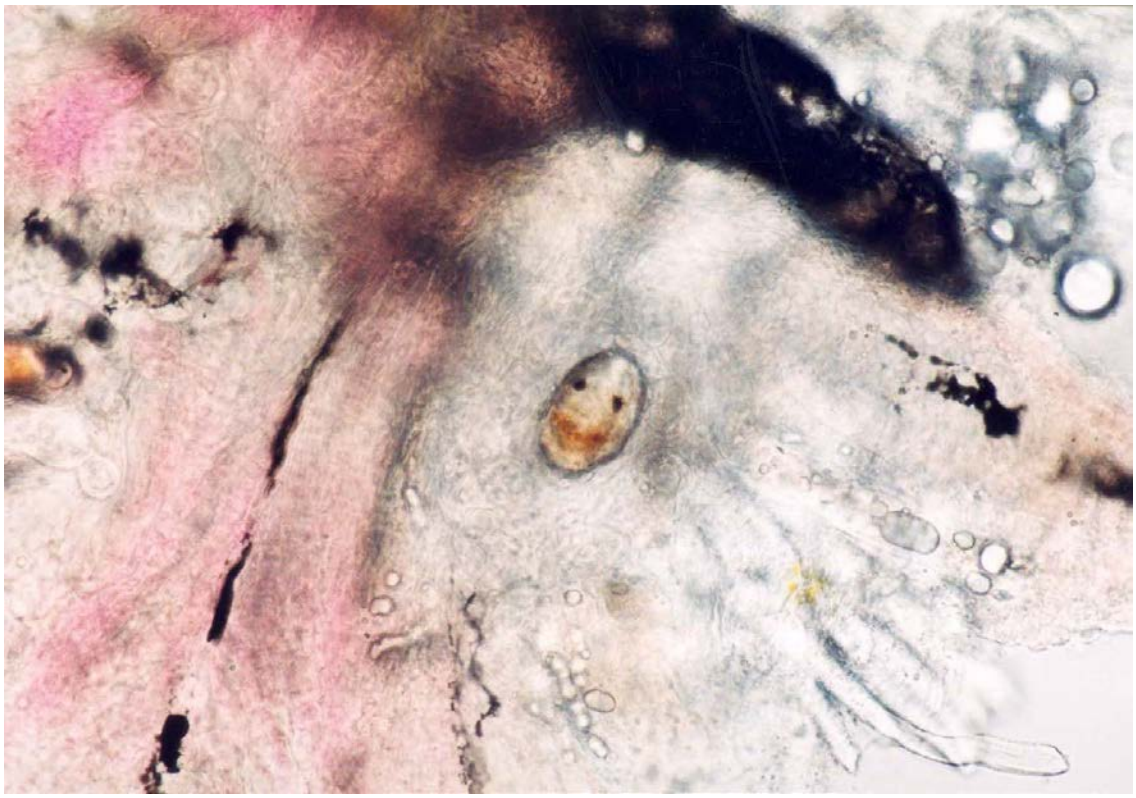

Figure 7. Dead metacercaria (parasite cyst about 95 μm in length) of *Centrocestus formosanus* encysted in the gills of a fountain darter (*Etheostoma fonticola*). Eye spots are still apparent.

The impact that this parasite has on the fish host depends on the number of metacercariae infecting the gill tissues and the size of the fish infected. Serious damage to the gills occurs in small darters (20 to 25 mm) infected with 25 cysts/gill arch while fat sleepers *Gobiomorus maculates* (up to 26 cm) survive infections of more than 350 cysts/gill arch). Also, individual fish species may have different host reactions that could affect the impact of the infection.

D. Disease Signs

1. Behavioral Changes Associated with the Disease

Increased respiration rates in experimentally infected fish have been observed. Infected fish may appear somewhat listless and lay on the bottom. Fish with a heavy gill trematode infection may die more readily following handling than fish with light infections or no infection at all.

2. External Gross Signs

No clinical signs are apparent when *C. formosanus* infections are light. In heavy infections, the opercular flaps, particularly of smaller fish, become flared by distorted branchial tissues. The gills may press against the wall of the pharynx and opercular flap producing an intense red color visible externally through the operculum (Figure 8).

Figure 8. Ventral view of a fountain darter (*Etheostoma fonticola*) with massive infection of *Centrocestus formosanus* and an uninfected fish (lower). Opercula flared outward by deformed and hyperplastic gill tissue (each division of scale = 1 mm).

3. Internal Signs

By light microscopy of gill wet mounts, cysts are evident in the gills (sizes given above) often with areas of clearing surrounding the cysts (Figure 9).

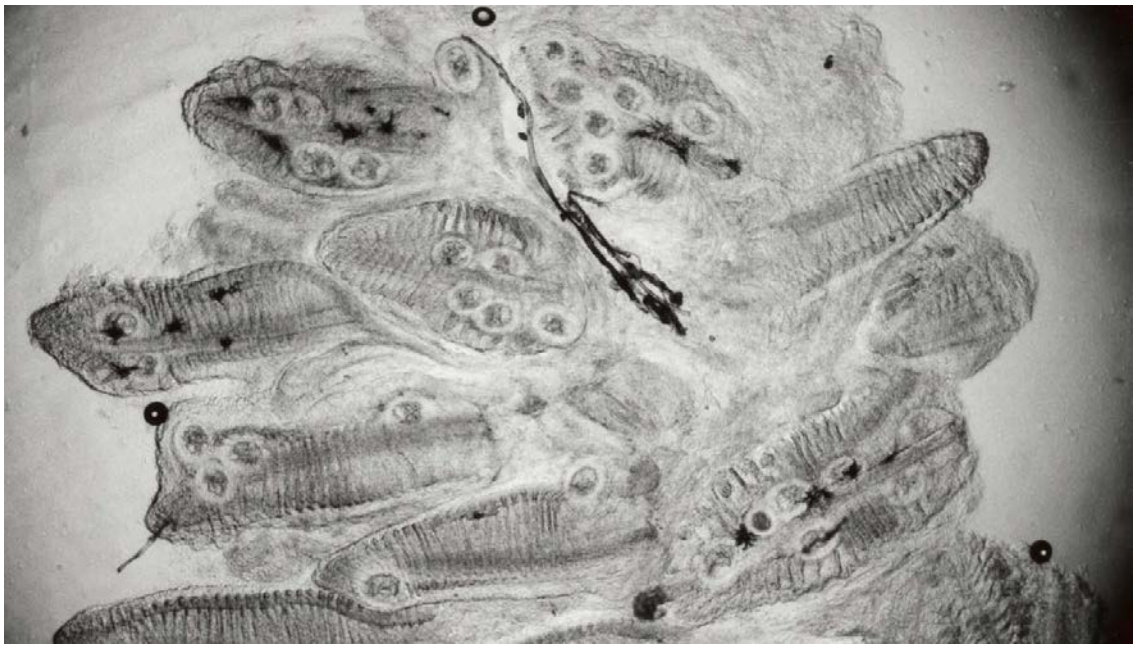

Figure 9. Gills of channel catfish (*Ictalurus puncatus*) experimentally infected with *Centrocestus formosanus*. Host cyst walls are seen as areas of clearing (cartilage proliferation) around metacercaria (parasite cyst about 100 μm in length).

In fish with numerous cysts, the filaments are shortened and thickened, there is epithelial hyperplasia, fusion of filaments, and lamellae engorged with red blood cells (Figure 10).

Figure 10. ntrocestus formosanus. Low and higher magnification views show shortened and thickened gill filaments, epithelial hyperplasia, and damaged lamellae engorged with blood (parasite cyst about 95 μm in length).

Gill cartilage is often deformed and may have thickened areas that bend or branch (Figure 11 video).

Figure 11 Video. Gill of a channel catfish (*Ictalurus puncatus*) experimentally infected with *Centrocestus formosanus.* Gill filaments are deformed (thickened and bent) in areas adjacent to parasite cysts.

4. Histopathological Changes Associated with the Disease

The trematodes migrate to just outside the cartilage rod and are encysted in chondrocytes and cartilage closely associated with the filamental cartilage. These cysts produce the clear areas seen in wet mounts of gill tissue. Distortion of the gill architecture frequently leads to a loss of lamellae in areas adjacent to the encysted parasite (Figure 12).

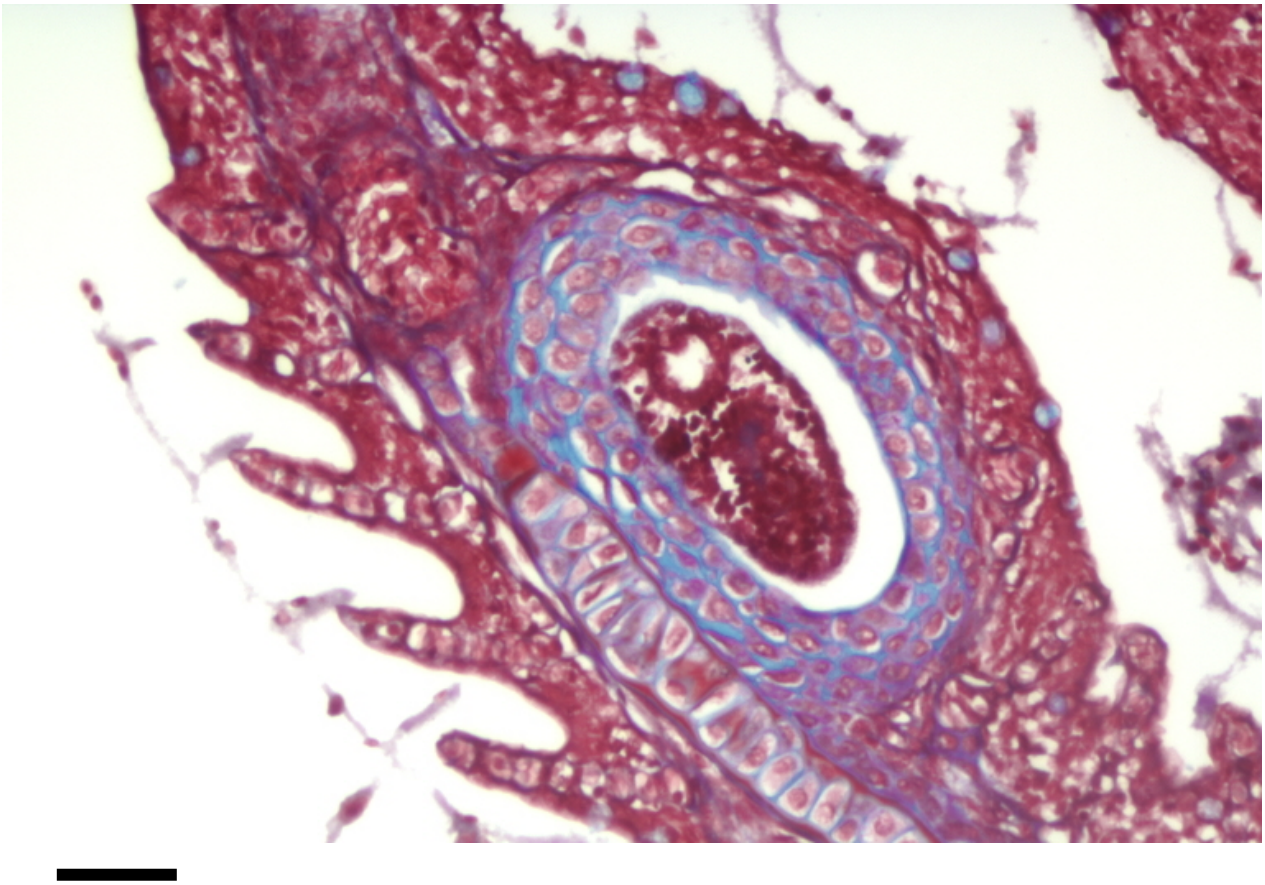

Figure 12. Histological section of a gill filament from a sunshine bass (*Morone chrysops* x *M. saxatilis*), experimentally infected with *Centrocestus formosanus* (Masson's trichrome stain). The host cyst wall stains blue, demonstrating that it is composed of cartilage. Lamellae adjacent to the cyst are lost. Scale bar = 100 μm.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

In wet mounts of gill tissue, the presence of a 75 to 225 µm ovoid cyst containing an organism with two eye spots (young metacercariae) or a dark X-shaped structure (mature metacercariae) provides presumptive evidence of the presence of *Centrocestus formosanus*.

2. Confirmatory Diagnosis

There are no definitive techniques available for the diagnosis of *C. formosanus*; specific PCR methods are under development, but not yet sufficiently validated for use. Centrocestiasis is therefore confirmed by a combination of morphometric characters and a few distinctive pathological features associated with the infection. Under a dissection scope the cysts are teased from the gill tissue with a scalpel and using two scalpels (#11 blades) the metacercariae can be freed from the cyst. Coverslip pressure will sometimes free the worms from the cysts. When liberated from the cyst the metacercariae take a pyriform or conical shape and the body is entirely covered with tegumental spines (very small spines seen on the periphery of the worm – 100 to 400X) (Figure 13 video).

Figure 13 Video. Metacercaria of *Centrocestus formosanus* removed from cysts. Tegumental spines are seen along the periphery of the parasite.

In mature metacercariae, in addition to having X-shaped excretory bladders, two offset rows of 16 circumoral spines (total = 32 spines) can be counted. Further characterization using morphometric and meristic characters can be made by a parasitologist. Some distinctive pathological features include the distortion of gill cartilage and the presence of broad, clear-walled cysts surrounding the metacercariae. Histological confirmation that the host tissue cyst wall is composed of chondrocytes is important. Additional evidence may be gained by collecting red-rimmed melania from suspect waters and observing them for the release of cercariae that are about 230 to 300 µm total length with straight non-bifurcated tails and two prominent eye spots. Place five to ten snails in about 100 mL of water (20 to 30°C) and leave in the dark overnight, remove and expose to bright light for one hour, and examine water at 20 to 100X for cercariae.

The only other trematode species infecting freshwater fish in the USA that could be described with an X-shaped excretory bladder and circumoral spines are *Ascocotyle spp*., but they are not found encysted in the gills. In one case, trematodes in cartilaginous cysts were found in the gill filaments of steelhead trout *Oncorhynchus mykiss* caged in the Willamette River in Oregon. These trematodes were reported to have only one row of circumoral spines and therefore thought not to be *C. formosanus*.

F. Procedures for Detecting Subclinical Infections

Fish located in waters in USDA Plant Hardiness Zones of 9a or higher (usna.usda.gov/Hardzone/ushzmap.html) have the potential to be infected with this trematode. These areas are found in the southern parts of Florida, Texas, Arizona, Nevada and California. Also, fish in rivers that originate from springs or geothermal water with year round temperatures between 17 and 32°C are also candidates for infection especially if red-rimmed melania are found in the water. There

is potential for the infection to occur in fish in waters that are heated by industrial effluents year round.

To detect subclinical infections in fish, the second or third gill arch should be removed in its entirety and, if the fish is less than 40 mm, put on a glass slide covered with a coverslip and viewed under a dissection scope or binocular scope at 20X. For fish larger than 40 mm, the cartilaginous gill arch will keep the coverslip from flattening the gills and trematodes may remain hidden. Therefore, carefully remove the filaments from the arch for viewing. Any ovoid objects around 75-225 μm in diameter should be further examined at higher powers (100 to 400X) to determine if characteristic cysts and metacercariae are present. Suspect tissues may be fixed in 10% formalin for later confirmation by histology and in 70 to 95% ethanol for light microscopy.

G. Procedures for Determining Prior Exposure to the Etiological Agent

Cyst elimination may begin 50 days post infection and probably will be complete within a year. There may be residual thickening, branching or bending of the filament cartilage but other parasites, including at least one myxosporian, may leave similar lesions. There is no conclusive method for determining the presence of prior infections if the parasite has been eliminated.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival or Recognition of the Etiological Agent

Submitting live fish is best; if this is not possible, put fish on ice and ship to a diagnostic laboratory within 48 h. As a last resort put specimens in 70 to 95 % ethanol (if the fish is large, remove and fix the second or third gill arch). For histology, fix fish in 10% formalin.

References

Alcaraz, G., P. de Leon, P. Garcia, V. Leon-Regagnon, and C. Vanegas. 1999. Respiratory responses of grass carp *Ctenopharyngodon idella* (Cyprinidae) to parasitic infection by *Centrocestus formosanus* (Digenea). The Southwestern Naturalist 44(2):222-226.

Amaya-Huerta, D., and R. J. Almeyda-Artigas. 1994. Confirmation of *Centrocestus formosanus* (Nishigori, 1924) Price, 1932 (Trematoda: Heterophyidae) in Mexico. Research and Review in Parasitology 54(2):99-103.

Kagei, N., and Y. Yanohara. 1995. Epidemiological study on *Centrocestus formosanus* (Nishigori, 1924) – survey of its infection in Tanegashima, Kagoshima Prefucture, Japan. Japanese Journal of Parasitology 44(2):154-160.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes, Second Edition. Comstock Publishing Associates, Cornell University Press, Ithaca, New York. 539 pp.

Lo, C. -T., and K. -M. Lee. 1996. Infectivity of the cercariae of *Centrocestus formosanus* and *Haplorchis pumilio* (Digenea: Heterophyidae) in *Cyprinus carpio*. Zoological Studies 35(4):305-309.

Lo, C. -T., and K. -M. Lee. 1996. Pattern of emergence and the effects of temperature and light on the emergence and survival of heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis*

pumilio). Journal of Parasitology 82(2):347-350.

Mitchell, A. J., M. J. Salmon, D. G. Huffman, A. E. Goodwin, and T. M. Brandt. 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. Journal of Aquatic Animal Health 12:283-289.

Mitchell, A. J., A. E. Goodwin, M. J. Salmon, and T. M. Brandt. 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestsus formosanus*, in four aquaculture fishes. North American Journal of Aquaculture 64:55-59.

Nishigori, M. 1924. On a new trematode *Stamnosoma formosanum* n. sp. and its life history. Taiwan Igakkai Zasshi 234:181-228.

Olsen, R. E. 1997. A trematode metacercaria causing gill cartilage proliferation in steelhead trout from Oregon. Journal of Wildlife Diseases 33(4):886-890.

Premvati, G., and V. Pande. 1974. On *Centrocestus formosanus* (Nishigori, 1924) Price, 1932 and its experimental infection in white Leghorn chicks. Japanese Journal of Parasitology 23(3):79-84.

Salgado-Maldonado, G., M. I. Rodriguez-Vargas, and J. J. Campos-Perez. 1995. Metacercariae of *Centrocestus formosanus* (Nishigori, 1924) (Trematoda) in freshwater fishes in Mexico and their transmission by the thiarid snail *Melanides tuberculata*. Studies on Neotropical Fauna and Environment 30(4):245-250.

Scholz, T., and G. Salgado-Maldonado. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae) in Mexico: a review. The American Midland Naturalist 143(1):185-200.

Wailagul, J. 1998. *Opisthorchis viverrini* metacercaria in Thai freshwater fish. Southeast Asian Journal of Tropical Medicine and Public Health 29(2):324-326.

Wilson, C. 2003. Another new exotic fish pathogen comes to Utah. The Ichthyogram 14(1):5.

Wilson, C. 2003. *Centrocestus* parasite discovered at second location in Utah. The Ichthyogram 14(2):5.

Yamaguti, S. 1975. A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates: with Special Reference to the Morphology of Their Larval Forms. Kaigaku Publishing Co., Tokyo, Japan. 590 pp.

Yanohara, Y., H. Nojima, and A. Sato. 1987. Incidence of *Centrocestus formosanus* infection in snails. The Journal of Parasitology 73(2):434-436.

Yanohara, Y., and N. Kagei. 1983. *Centrocestus formosanus* (Nishigori, 1924). Fish Pathology 17(4):237-241.