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Second Intermediate Host-Specificity of *Haematoloechus complexus* and *Haematoloechus medioplexus* (Digenea: Haematoloechidae)

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ABSTRACT: Second intermediate host-specificity was examined for 2 species of the frog lung fluke genus *Haematoloechus*. Nine species of freshwater arthropods were exposed to cercariae of *H. complexus* and *H. medioplexus*. Metacercariae of *H. complexus* developed in all arthropod species used. Metacercariae of *H. medioplexus* developed only in anisopteran odonate naiads. This difference in host utilization may have epizootiological implications. The potential development of *H. complexus* in a greater number of arthropods than *H. medioplexus* may increase the chances of ingestion of *H. complexus* by an anuran host. The range of arthropods parasitized by *H. complexus* indicates that host-specificity of the metacercarial stage of this fluke is not restricted by the phylogeny of aquatic arthropods.

Frog lung flukes of the genus *Haematoloechus* are generally considered to utilize only odonates as second intermediate hosts. For example, *H. varioplexus* encysts within members of the dragonfly genus *Sympetrum* in North America (Krull, 1931) and *H. johnsoni* is present in African anisopterans (Bourgat and Kulo, 1979). A few species of *Haematoloechus* have also been found to parasitize zygopterans. *Haematoloechus longiplexus* was reported from *Lestes vigilax* in Michigan by Krull (1932) and Grabda (1960) reported the European *H. similis* only from damselflies of the genus *Coenagrion*. Anisopteran and zygopteran odonates are distinguished on the basis of differing respiratory structures. Anisopteran (dragonfly) odonate naiads acquire oxygen by sucking water into the rectum, which has been modified as an organ of gas exchange. Zygopteran (damselfly) naiads utilize 3 external gills on the terminus of the abdomen for gas exchange.

Lung fluke metacercariae have rarely been found in non-odonate arthropods. *Haematoloechus variegatus* was reported by van Theil (1930) from larvae of the mosquito *Anopheles maculipennis*. Another species of *Haematoloechus* was found by Dollfus et al. (1960) to parasitize 2 species of the dipteran genus *Culex* as well as *A. maculipennis*. In addition, Combes (1968) experimentally infected 2 species of plecopteran larvae with *H. pyrenaicus*.

The present work examines experimentally the second intermediate host-specificity of 2 North American haematoloechids, *H. complexus* and *H. medioplexus*. Both parasites were obtained from the northern leopard frog *Rana pipiens* collected from Nevens Pond, a stock tank runoff pond in Keith County, Nebraska (SW ¼, Sec. 2, T14N, R36W). Within this pond, *H. complexus* uses *Physella virgata* as its first intermediate host, and *H. medioplexus* parasitizes the planorbid snail *Gyraulus parvus.*

Laboratory infections of *H. complexus* were successfully established in *Physella gyrina, P. heterostropha,* and *P. virgata,* and *Haematoloechus medioplexus* infections were established in *Promentus exacuous* and *G. parvus.* Adult flukes used in these infections were identified according to Kennedy (1981), and eggs of these parasites were differentiated on the basis of size. Eggs of *H. complexus* from the Nevens Pond population are approximately 33 μm long and eggs of *H. medioplexus* approximately 26 μm in length. Parasite eggs were harvested by placing single adult worms in jars containing aged tap water. The jars were covered and the eggs allowed to sit for at least 7 days. Laboratory-reared snails were then placed in the jars along with a small amount of Tetra Min® slurry to encourage the snails to consume the eggs. Eggs of *H. complexus* were found hatched in the feces of *G. parvus* and the previously mentioned physids. Cercariae of this parasite were released only from the physids. Similarly, eggs of *H. medioplexus* were found hatched in the feces of the physids as well as *G. parvus,* but this parasite produced cercariae only in *G. parvus* and *Pr. exacuous.*

The cercariae of the 2 species differed according to size as well as host origin. *Haematoloechus medioplexus* cercariae were much smaller than those of *H. complexus.* The former cercariae had a body length approximately 98 μm, whereas *H. complexus* were approximately 169 μm long (excluding the tail). The difference in size and host origin of cercariae from laboratory-infected snails allowed for identification of these parasite species when cercariae were collected from naturally infected snails from the Nevens Pond site.

Naturally and experimentally infected snails were individually isolated in 150-ml plastic jars and fed a Tetra Min® slurry ad libitum. Water containing cercariae of 1 species was removed from all jars with shedding snails, pooled, and divided into aliquots, the volume of which varied according to the number of snails actively shedding cercariae on the day of the experiment and the number of arthropods available for exposure. The use of this protocol resulted in the exposure of different arthropod species to different numbers of cercariae. At least 20 cercariae were present in each jar, although the precise number was not counted. An effort was made to ensure that approximately the same number of the 2 different species of cercariae was used. The arthropods listed in Table I were placed in 200-ml plastic jars along with the water containing the cercariae of 1 of the parasite species.

Arthropods used in these experiments came from a variety of natural populations. To ensure that these animals were not naturally infected, 10 individuals of each species were selected at the time of experimental exposure and dissected to check for the presence of metacercariae (T_{zero} control). In the case of *Libellula* sp., however, only 4 animals were dissected due to the small number of naiads collected. As an additional control, another group of 10 (4 in the case of *Libellula* sp.) was randomly selected from each group of arthropods and held in the laboratory in aged tap water (T_t control).

Between 5 and 8 days postexposure (depending on the heartiness of the different arthropods in the lab) both experimental and T_t control arthropods were dissected and examined for the presence of metacercariae. Not all arthropods survived to the date of dissection.

Metacercariae of *H. complexus* were found in individuals of all 9 arthropod species exposed to cercariae (Table I). These arthropods represent 2 subphyla and 3 insect orders. Following laboratory exposure, metacercariae of *H. medioplexus* were found only in the anisopteran odonates *Anax junius* and *Libellula* sp. In no instance were metacercariae of any species found in animals that served as controls. Table I lists the number of arthropods that harbored metacercariae and the number initially exposed to cercariae that survived to the dissection date.

Previous reports of second intermediate host utilization of *H. complexus* are limited to anisopteran and zygopteran odonates (Ingles, 1933; Krull, 1933; Dronen, 1975). The present study indicates that the metacercariae of *H. complexus* are generalists, able to utilize a much wider range of second intermediate hosts than previously believed. *Haematoloechus medioplexus,* however, was found to be a second intermediate host specialist, parasitizing only anisopteran odonates, a result consistent with earlier work (Krull, 1930, 1931).

A wide range of second intermediate host utilization by a generalist species might well increase the total number of infected arthropods in nature when compared to the number infected by a specialist. Thus, an anuran would have a better chance of ingesting a food item infected by the generalist than by the specialist. Members of the *R. pipiens* complex have been reported to eat ephemeropteran larvae, amphipods including *Gammarus lacustris,* chironomid midges, and a variety of adult and larval odonates (Drake, 1914; Knowlton, 1944; Kilby, 1945). It should be noted that some of the arthropods used in this study may never enter the diet of *R. pipiens.* For example, the mayflies *Leptophlebia* spp. generally occupy lotic habitats (Merritt and Cummins, 1978) where contact with *R. pipiens* is probably rare. What should be emphasized is the taxonomic range of arthropods susceptible to parasitism by *H. complexus.* This range indicates that *H. complexus* is likely able to parasitize almost any aquatic arthropod, including species of ephemeropterans that do co-occur with *R. pipiens.*

Field data lend tentative support to the idea that the generalist nature of *H. complexus* metacercariae may enable this parasite to infect more definitive hosts than the specialist *H. medioplexus. Haematoloechus complexus* had a higher prevalence than *H. medioplexus* in 18 *R. pipiens* collected from Nevens Pond between March and September 1993. These frogs ranged in size from 45 mm to 100 mm snout–vent length. *Haematoloechus complexus* was present in 13 out of 18 (72.2%) frogs, whereas *H. medioplexus* was present in only 6 of 18 (33.0%). This difference is statistically significant when the data are analyzed using chi-square in a 2×2 contingency table (chi-square = 5.46, df = 1, $P < 0.05$). Furthermore, the relative density of *H. complexus* (6.4 flukes/frog) is greater than that of *H. medioplexus* (1.33 flukes/frog). Relative density data were distributed non-normally and were thus ranked and analyzed using Student's *t*-test. This procedure indicated a significant difference between the relative density of *H. complexus* and *H. medioplexus* ($t = 5.24$, df = 34, $P < 0.05$). The difference between the mean intensity of *H. complexus* (8.9 flukes/infected frog) and *H. medioplexus* (4.0 flukes/infected frog) was not statistically significant when the data were ranked and analyzed using Student's *t*-test ($t = 1.88$, df = 17, $P < 0.1$).

The differences in prevalence and relative density could obviously be due to factors unrelated to second intermediate host utilization, for example differences in the size of first intermediate host populations or differences in anuran susceptibility to the parasites. However, these results do suggest the potential epizootiological importance of the relatively broad second intermediate host range of *H. complexus.*

TABLE I. Results of experimental exposures of arthropods to cercariae of *Haematoloechus complexus* and *H. medioplexus.*

	Number infected/number of exposed survivors	
Experimental arthropods	*Haematoloechus complexus*	*Haematoloechus medioplexus*
Insecta		
Odonata: Anisoptera		
Anax junius	8/8	7/9
Libellula sp.	3/3	3/3
Odonata: Zygoptera		
Enallagma civile	3/3	0/6
Ischnura verticalis	10/10	0/10
Ephemeroptera		
Leptophlebia sp.	6/6	0/9
Diptera: Chironomidae		
Eukiefferiella sp.	6/8	0/9
Crustacea		
Isopoda		
Asellus intermedius	8/10	0/10
Asellus militaris	6/10	0/10
Amphipoda		
Gammarus lacustris	5/6	0/9

Combes (1968) found metacercariae of *H. pyrenaicus* encysted only within the respiratory organs of both plecopteran larvae and anisopteran naiads. He speculated that the ability of *H. pyrenaicus* to parasitize both types of insects was related to the similar nature of gill structure among these 2 animals. Thus, similarity of encystment sites was thought to be more important than host phylogeny in determining host suitability. In the present study, *H. medioplexus* metacercariae were found only in the branchial baskets of anisopteran hosts. *Haematoloechus medioplexus* was unable to infect other gill-bearing arthropods. Metacercariae of *H. complexus,* however, were found throughout the bodies of infected arthropods, encysting in the heads, thoraces, and abdomens, as well as within the appendages of many second intermediate hosts. *Haematoloechus complexus* metacercariae did encyst within the gills of anisopteran odonate naiads and freshwater isopods but were also present throughout the rest of the bodies of these animals. The broad taxonomic range of second intermediate hosts utilized by *H. complexus* indicates that host phylogeny is not a dominant factor in determining suitability among aquatic arthropods for this parasite species.

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