Suspending Life Cycles in the Cold: Survival of Larval and Cyst Stages of Gordiids (Nematomorpha) to Freezing.

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ABSTRACT

Horsehair worms are parasites of terrestrial arthropods, but are free-living in aquatic environments as adults. At the end of their parasitic stage, gordiids manipulate the behavior of their arthropod hosts to enter an aquatic environment, were they mate, and reproduce. Females produce millions of eggs that develop into larvae which infect and encyst within a variety of aquatic invertebrates. Some of these infected animals act as transport hosts by carrying cysts to land where they are consumed by crickets. Although gordiids have often been called one of the most understudied groups of animals, recently a number of species are being cultured in our laboratories. However, one set back in maintaining gordiid life cycles in culture is the enormous amount of time needed to rear and maintain the multiple species of hosts for the worms to develop in. Therefore, we explored the possibility of pausing the life cycle of these worms by freezing the larval and cyst stages of two species of North American and African horsehair worms at low temperatures (-80°C). Data from our study suggest that both North American and African hairworm species survive freezing during the larval and cyst stages in water and are infective to their snail and cricket hosts respectively. This ability to withstand freezing is considered an adaptation in some invertebrates to living in cold environments, however it is unclear what advantage the ability to freeze at low temperatures has for gordiids.

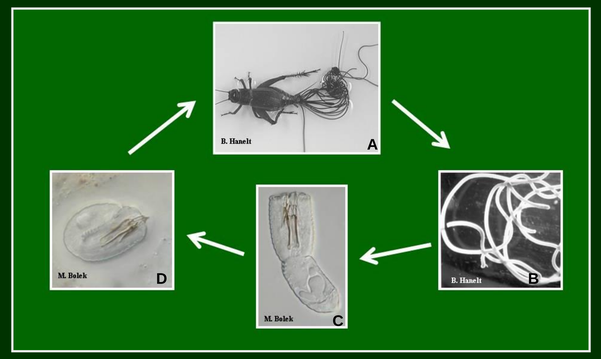

Fig. 1 (A) Cricket definitive host releasing free living worms; (B) eggs strings; (C) free living larvae; (D) mature infective cyst from snail.

Question

Can North American and African gordiid larval and cyst stages survive freezing at -80˚ C?

METHODS

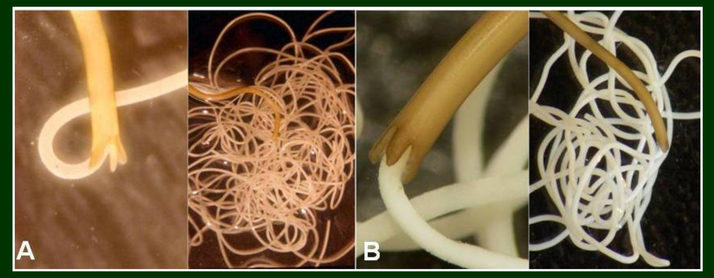

Fig. 2 Species of horsehair worms tested (A) North American *Paragordius vairus* female laying eggs; (B) African parthenogenic *Paragordius* n. sp. female laying eggs.

EXPERIMENT I

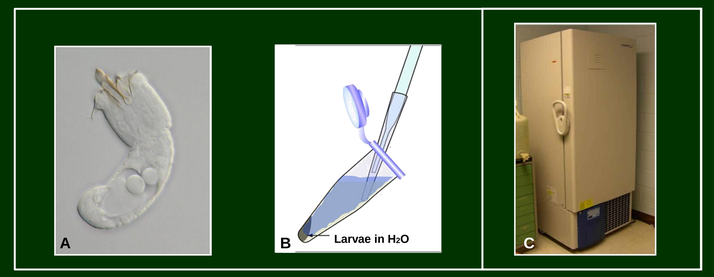

Fig. 3 (A) Infective larva; (B) larvae placed in eppendorf tubes in H_2O; (C) larvae in H_2O frozen at -80 °C for 8 months.

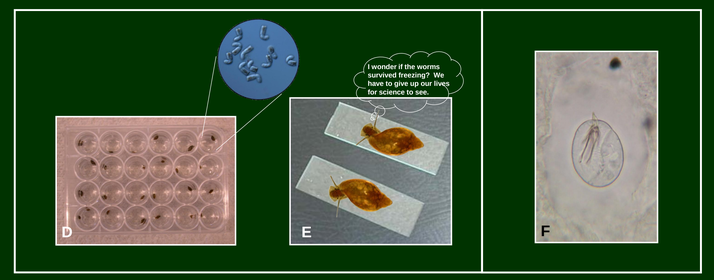

Fig. 4 (D) Snail infections with defrosted larvae; (E) snail necropsy; (F) cyst count.

EXPERIMENT II

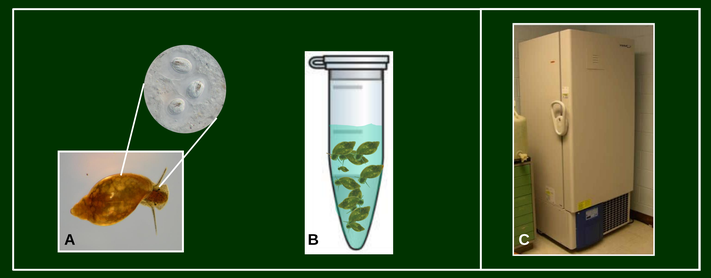

Fig. 5 (A) Infected snail with cysts; (B) snails with cysts placed in eppendorf tubes in H_2O; (C) snails with cysts in H_2O frozen at -80 °C for 8 months.

Fig. 6 (D) Cricket infections with defrosted cysts in snail tissue; (E) cricket releasing worms in water; (F) adult female laying eggs.

RESULTS

Table I. Results of experimentally infected snails and crickets with defrosted *P. varius* and *Paragordius* n. sp. larvae or cysts.

Hairworm Species	No. Surviving/No. Exposed	Prevalence	Mean Intensity ± 1SD
Paragordius varius			
Snails	20/20	100%	61 ± 49
Crickets	14/60	65%	5.1 ± 5.1
Paragordius n. sp.			
Snails	20/20	100%	114 ± 98
Crickets	6/20	17%	1

CONCLUSION

Our results indicate that both larval and cyst stages of North American and African gordiids can survive freezing at -80 °C , and both stages are infective to snail and cricket hosts respectively.