

## Introduction

The microsporidian parasite *Cougourdella* sp. (Fig. 1A) is known to infect populations of the caddisfly *Glossosoma nigrior* (Trichoptera: Glossosomatidae; Fig. 1B) in coldwater (trout) streams in Michigan and Maine. Infections invariably result in host death before hosts reach the adult stage. *Glossosoma* is a strong interactor in trout stream ecosystems, and has pronounced effects on food web structure and function (McAuliffe 1984, Kohler 1992). Where present, *Cougourdella* has keystone effects on food web structure through its impacts on *Glossosoma* population dynamics (Kohler and Wiley 1997) (Fig. 2). Whether such effects occur more generally is unknown in part because *Cougourdella*'s distribution has not been described. Our objectives were to survey coldwater streams in the eastern United States from Georgia to Vermont to determine: 1) if *Cougourdella* occurs throughout the range of *Glossosoma nigrior*, 2) if microsporidiosis in *Glossosoma* is produced by a single species of *Cougourdella*, and 3) whether disease epizootics occur in streams outside of Michigan and Maine.

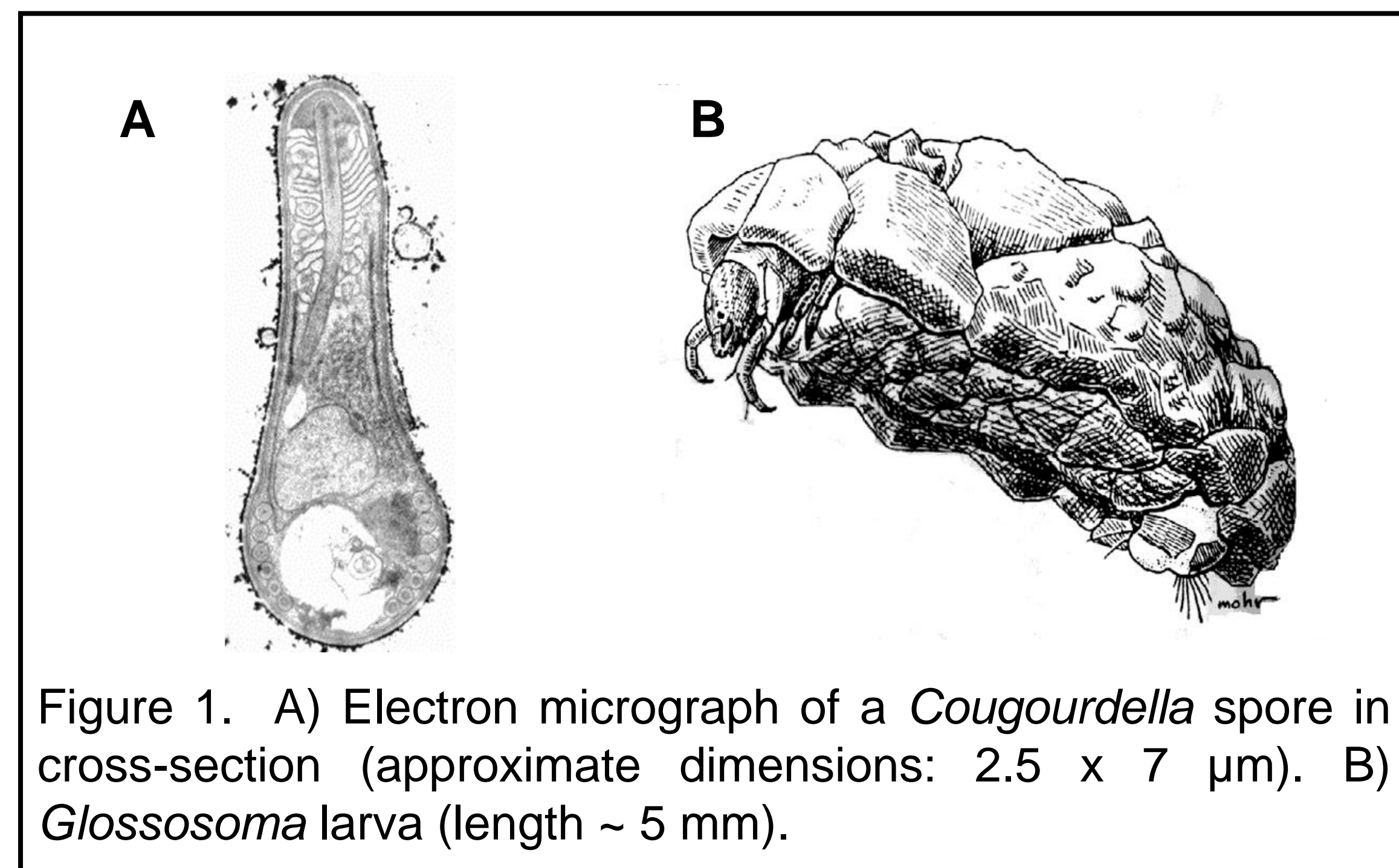


Figure 1. A) Electron micrograph of a *Cougourdella* spore in cross-section (approximate dimensions: 2.5 x 7  $\mu$ m). B) *Glossosoma* larva (length ~ 5 mm).

### *Cougourdella* epizootics regulate *Glossosoma* dynamics

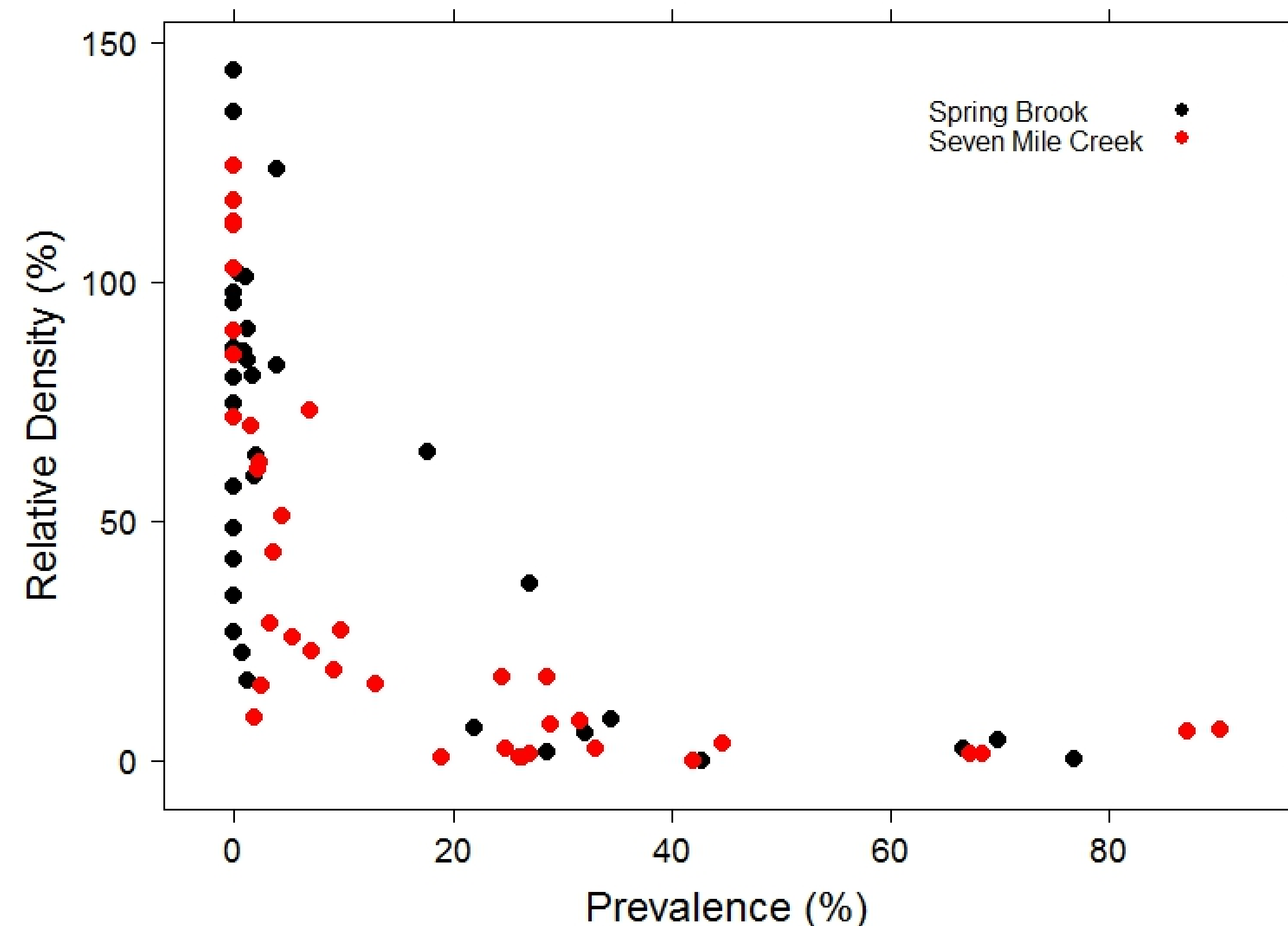


Figure 2. *Glossosoma* density, expressed relative to the mean pre-collapse density, as a function of disease prevalence for two streams in southwest Michigan, based on > 15 year time series for each stream. Each data point represents 1 *Glossosoma* generation. Piecewise linear regression (using SiZer in R) suggests prevalence  $\geq 10\%$  (95% CI = 2,30) is sufficient to maintain populations at low density.

# Distribution and Prevalence of a Keystone Parasite in the Eastern United States

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## Methods

We attempted to collect *G. nigrior* larvae and pupae from five streams in Georgia, 14 in Virginia, 17 in Pennsylvania, and 10 in Vermont. At each site we tried to collect 100 individuals. All individuals collected were preserved immediately in 70% ethanol.

To screen for *Cougourdella* infection, individuals were homogenized in ~ 0.5 mL distilled water in 1.5 mL centrifuge tubes. Homogenates for each individual were examined for infection using phase contrast microscopy at 400X. We first screened 10 individual larvae. If no infections were detected, the remaining larvae were screened in groups of two (i.e., 2 larvae per centrifuge tube). Larvae and pupae were tested separately because disease prevalence is often lower in pupae. *Cougourdella* prevalence in each *G. nigrior* population was estimated using procedures described in Hoenig et al. (1987).

We used DNA sequencing of the SSU rDNA gene to determine if the same species of *Cougourdella* was present in *G. nigrior* populations in the eastern United States as in Michigan. DNA was extracted from *Cougourdella* spores from isolates obtained in Georgia, North Carolina, and Pennsylvania, amplified by PCR using microsporidian-specific primers, and gel-extracted PCR products were sequenced at the University of Michigan DNA Sequencing Core.

## Results

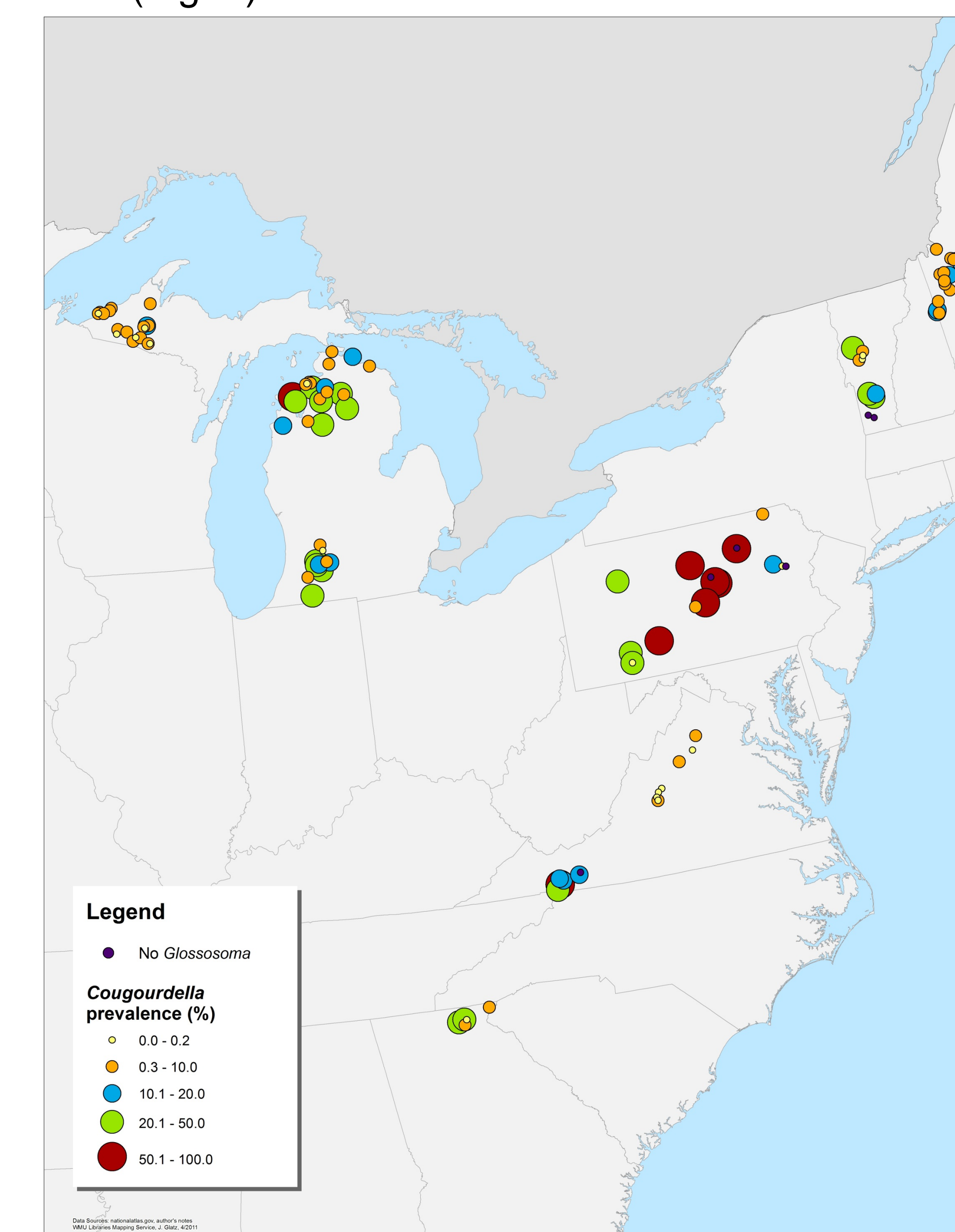
SSU rDNA gene sequences from Georgia, North Carolina, Pennsylvania and Michigan *Cougourdella* isolates were nearly identical (> 98% similarity), which suggests that a single *Cougourdella* species infects *G. nigrior* populations in the eastern United States.

*Cougourdella* was present in streams supporting *Glossosoma nigrior* populations in all regions surveyed (Table 1). Overall, 75.6% of *G. nigrior* populations were infected by *Cougourdella*. The proportion of populations infected was very similar for the subset of streams where  $\geq 10$  *G. nigrior* were collected (74.2%; Table 1).

>Table 1. The number of streams sampled in each state in which *Glossosoma* was found, the number of streams in which at least 10 *Glossosoma* individuals were collected, and the number of those streams in which *Cougourdella* was detected.

State	Streams with <i>Glossosoma</i>		Streams with <i>Cougourdella</i>	
	All	$\geq 10$ <i>Glossosoma</i>	All	$\geq 10$ <i>Glossosoma</i>
Georgia	5	5	4	4
Virginia	13	9	8	6
Pennsylvania	15	11	13	9
Vermont	8	6	6	4

*Cougourdella* prevalence in *G. nigrior* populations ranged from 0 to 100% (Fig. 3). Disease prevalence was  $\geq 20\%$  in 15 of 31 streams (48.4%) in which *Cougourdella* was present. *G. nigrior* populations supporting high disease prevalence were present in all regions surveyed (Fig. 3). For comparative purposes, mean disease prevalence in Michigan and Maine streams that have been surveyed for > 10 years is shown in Figure 3. The incidence of disease in Michigan and Maine *G. nigrior* populations (62 of 70 streams; 88.6%) and prevalence levels are quite similar to those observed in the streams surveyed in 2009 (Fig. 3).



>Figure 3. Incidence and prevalence of *Cougourdella* in *Glossosoma nigrior* populations.

## Conclusions

>The microsporidian parasite *Cougourdella* sp. is associated with *Glossosoma nigrior* populations throughout *G. nigrior*'s range in the eastern United States.

>Available evidence suggests that a single species of *Cougourdella* is responsible for disease epizootics in *G. nigrior* populations.

>Nearly 50% of infected *G. nigrior* populations exhibited relatively high disease prevalence. This suggests that disease epizootics are common, and that *Cougourdella* likely has keystone effects on stream food web structure throughout the eastern United States.

## Future Research

>We will apply pyrosequencing techniques to the *Cougourdella* genome to search for microsatellite markers.

>Candidate markers will be screened and a set of markers will be used to determine the genetic structure of *Cougourdella* populations throughout *G. nigrior*'s range in the eastern United States.

>Analysis of *Cougourdella* population genetic structure over this broad spatial scale should help to answer questions about the history of the host-parasite relationship and *Cougourdella*'s life cycle.

## References

- Hoenig, J., M. D. Heisey, M. W. Lawing, D. and D. Schupp, H. 1987. An indirect rapid methods approach to assessment. *Canadian Journal of Fisheries and Aquatic Sciences* 44:324-338.
- Kohler, S. L. 1992. Competition and the structure of a benthic stream community. *Ecological Monographs* 62:165-188.
- Kohler, S. L. and M. J. Wiley. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. *Ecology* 78:2164-2176.
- McAuliffe, J. R. 1984. Resource depression by a stream herbivore: effects on distributions and abundances of other grazers. *Oikos* 42:327-333.

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