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Microsporidia Infection in *Neochetina eichhorniae* and *N. bruchi* Populations in Texas

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PURPOSE: This technical note discusses the collection, dissection, and screening of *Neochetina bruchi* Hustache and *N. eichhorniae* Warner from sites in Texas for the infectious protozoan disease, microsporidiosis. The causal organisms (Microsporidia) have been shown to severely limit the effectiveness of the weevil agents by reducing egg production and shortening life spans (Center and Rebelo 2001). Surveys conducted in 1995 determined that microsporidia were not present in weevils collected from sites in Texas. During the summer of 2002, researchers from the U.S. Army Corps of Engineers, Engineering Research and Development Center (ERDC) again surveyed sites in Texas in an effort to assay *Neochetina* populations for the presence/absence of microsporidia. It is essential that infection of *N. bruchi* and *N. eichhorniae* be assessed so that impact from microsporidiosis can be evaluated. In addition, it is becoming commonplace to import weevils from sites outside of Texas to augment existing weevil populations. Such importations are risky if imported weevils have levels of infection higher than existing Texas populations.

BACKGROUND: In the early 1970's, *N. eichhorniae* and *N. bruchi* were released in the United States as biological control agents for waterhyacinth, *Eichhornia crassipes* (Mart.) Solms.-Laubach (Center et al. 1990, Grodowitz et al. 1997). In conjunction with *Niphograpta albiguttalis* (Warren) (=*Sameodes albiguttalis*), the weevils have been successful in reducing plant height and stature as well as reducing flowering and seed set. In some instances, large reductions in biomass have been observed; however, in other cases the biocontrol agents are producing little, if any, overall impact. These observations have prompted researchers to examine factors that might explain the variation in agent effectiveness.

One such possible factor may be the presence of microsporidia in *Neochetina* populations. Weevils used for the first releases received only limited screening for microsporidia and it was presumed that these initial introductions were disease free. It was only recently that microsporidia infection was detected in U.S. weevil populations in Florida. With the detection of high microsporidia infection rates at various Florida sites and the discovery of the disease's potential debilitating effect, researchers have begun more detailed studies.

It was in the late 1940's that microsporidia were first identified in insect biological control agents. Although microsporidia will not prevent establishment, the presence of microsporidia in insects can produce extensive damage to a colony (Kluge and Caldwell 1992) and often compromises the overall fitness of the insect (Center and Rebelo 2001). Microsporidiosis is a chronic disease that often strikes if the insects are stressed, rendering them more susceptible to a decline in health. The disease is usually transmitted by spore ingestion, copulation, or inoculation by parasites. It can also be transmitted via transovarian transmission (i.e., via the female's eggs). Once the disease has invaded the insect it begins to spread within the tissues and organs. Insects infected with the disease are lethargic and exhibit abnormal feeding behavior. The disease retards larval development and results in deformed pupae and adults, arrested growth and development, reduced longevity, and even

mortality. Depending on the climate and the number of spores ingested, generation time of microsporidia can be as short as 93 hr or as long as 40 days (Kluge and Caldwell 1992).

Recent studies indicate that *N. eichhorniae* generally have higher levels of microsporidia than *N. bruchi* (Center and Rebelo 2001). While *N. bruchi* harbors only a single species of microsporidia, *N. eichhorniae* is possibly infected by two species. In *N. eichhorniae* the infections are typically systemic, affecting the mid-gut, Malphigan tubules and the fat body. However, in *N. bruchi* the infections are usually light with spores found only in the mid-gut. Weevils infected with microsporidia usually do not exhibit external symptoms that are easily recognizable. The only reliable method of detection is by microscopic examination (Kluge and Caldwell 1992).

Materials and Methods: Two different methods were used to detect the presence of microsporidia in populations of weevils collected in Texas. Both methods require a detailed examination of internal organs from live insects. In 1995 collections, the weevils were examined by using the "crushing method"; e.g., live weevils were crushed in a small amount of distilled water using a tissue grinder. The crushed insects were smeared onto a glass microscope slide using circular movements and allowed to dry for 2 hr. The dried specimens were then immersed in 100 percent methanol. After 5 min the slides were carefully shaken to remove excess methanol and stained with Giemsa stain. The crushed tissues were examined under 40X magnification, by using phase-contrast microscopy. Although this procedure can screen more than one insect at a time, it is not the most accurate. Singly examining the internal organs of living insects has been found to be the most accurate; therefore, this procedure was employed in the 2002 surveys.

In 2002, internal organs within the abdominal cavity of individual weevils were carefully removed using standard dissection techniques. The dissected organs were placed onto a glass microscope slide in a small quantity of distilled water. A No. 1 cover slip was placed over the tissue sample to protect the dissected organs during microscopic examination. The tissue sample was then carefully examined under 40X magnification using phase-contrast microscopy. Microsporidia spores appear blue with a glowing ring surrounding the spores due to the refraction from the phase-contrast illumination (Figure 1).

RESULTS AND DISCUSSION: Over 2,900 weevils were hand collected in 1995 from seven sites in Texas and screened for microsporidia (Figure 2). The disease was apparently absent in Texas at that time because microsporidia spores were not observed in any of the examined specimens. During the summer of 2002, 600 weevils were hand collected from eight sites in Texas and singly examined for microsporidia (Figure 2). Less than 1 percent or only four insects were infected with microsporidia; i.e. three *N. eichhorniae* weevils and one *N. bruchi*. All four infected weevils were females. Infected weevils were present at three of the eight sites or 40 percent of the water bodies surveyed in 2002 (Figure 3).

The percentage of microsporidia infection in female weevils was minimal, being only 1.43 percent. The overall combined total rate of male and female infection was only 0.67 percent. At BA Steinhagen, only one female *N. eichhorniae* was infected out of 25 examined, resulting in a 4-percent infection rate, but the combined percent for both males and females was only 1 percent (Figure 3). Two out of 43 N. eichhorniae females were infected in the Lake Conroe population, resulting in an infection rate of 4.65 percent. The combined rate of infection was 2.30 percent. Wallisville had the highest microsporidia infection rate for females at 5.26 percent where one of 19

female *N. bruchi* females was infected; however, the overall infection rate at this site was only 1.12 percent.

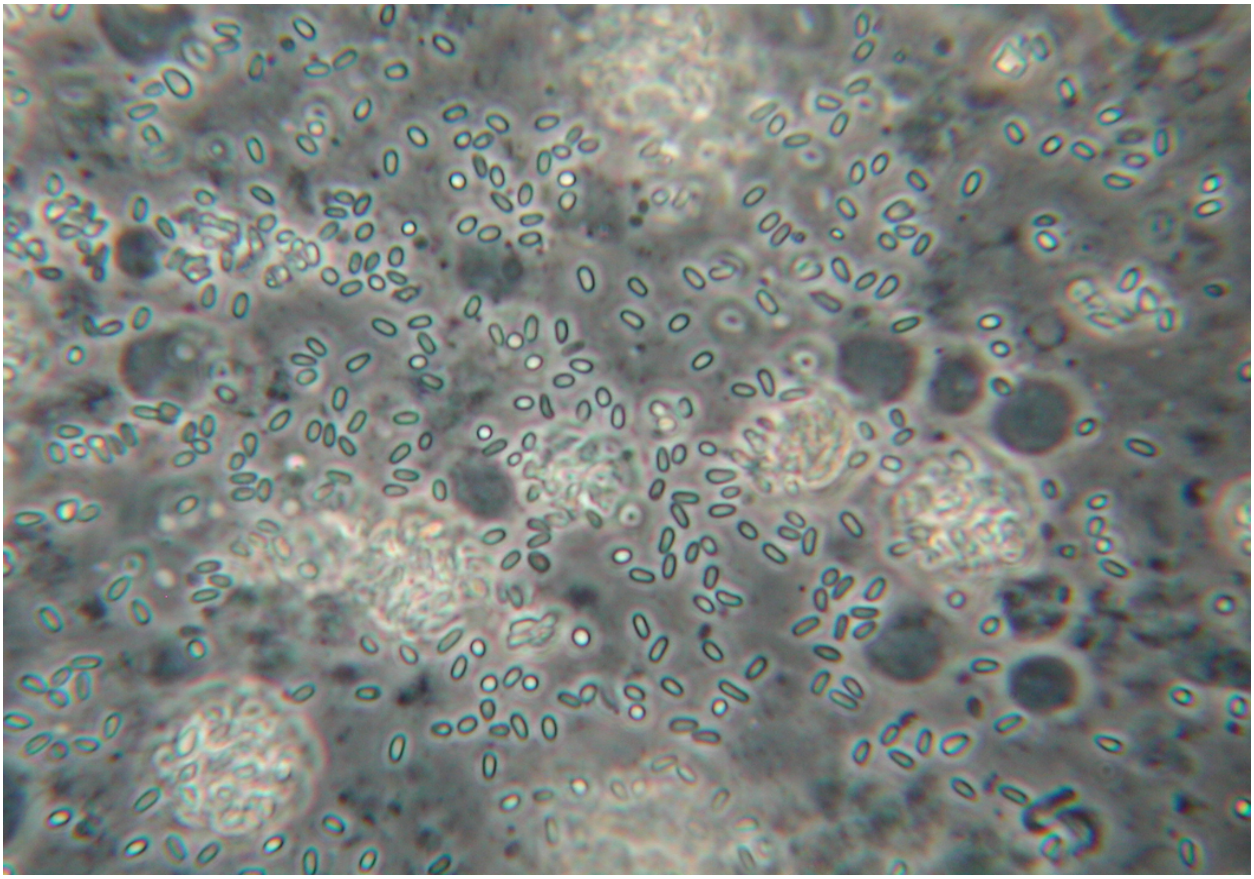

Figure 1. Microsporidia spores appear oblong in shape and occasionally clump together. They appear blue with a glowing ring surrounding the spores due to the refraction from the phase-contrast illumination (Photo taken by Teresa Rebelo)

Generally, infection of *Neochetina* spp. in Texas is relatively low with overall infection rates never exceeding 1.5 percent for females and less than 1 percent overall. This is in contrast to some sites in Florida where infection rates exceed 25 percent (unpublished data). Hence, microsporidia impacts should be low at most sites in Texas. However, this represents a relatively small sample size as only a few sites were examined. To increase sample size, more detailed sampling at a broader range of sites throughout the growing season should be initiated. Only limited information is available that describes changes in infection through time, and infection rates may change significantly as population numbers increase later in the growing season.

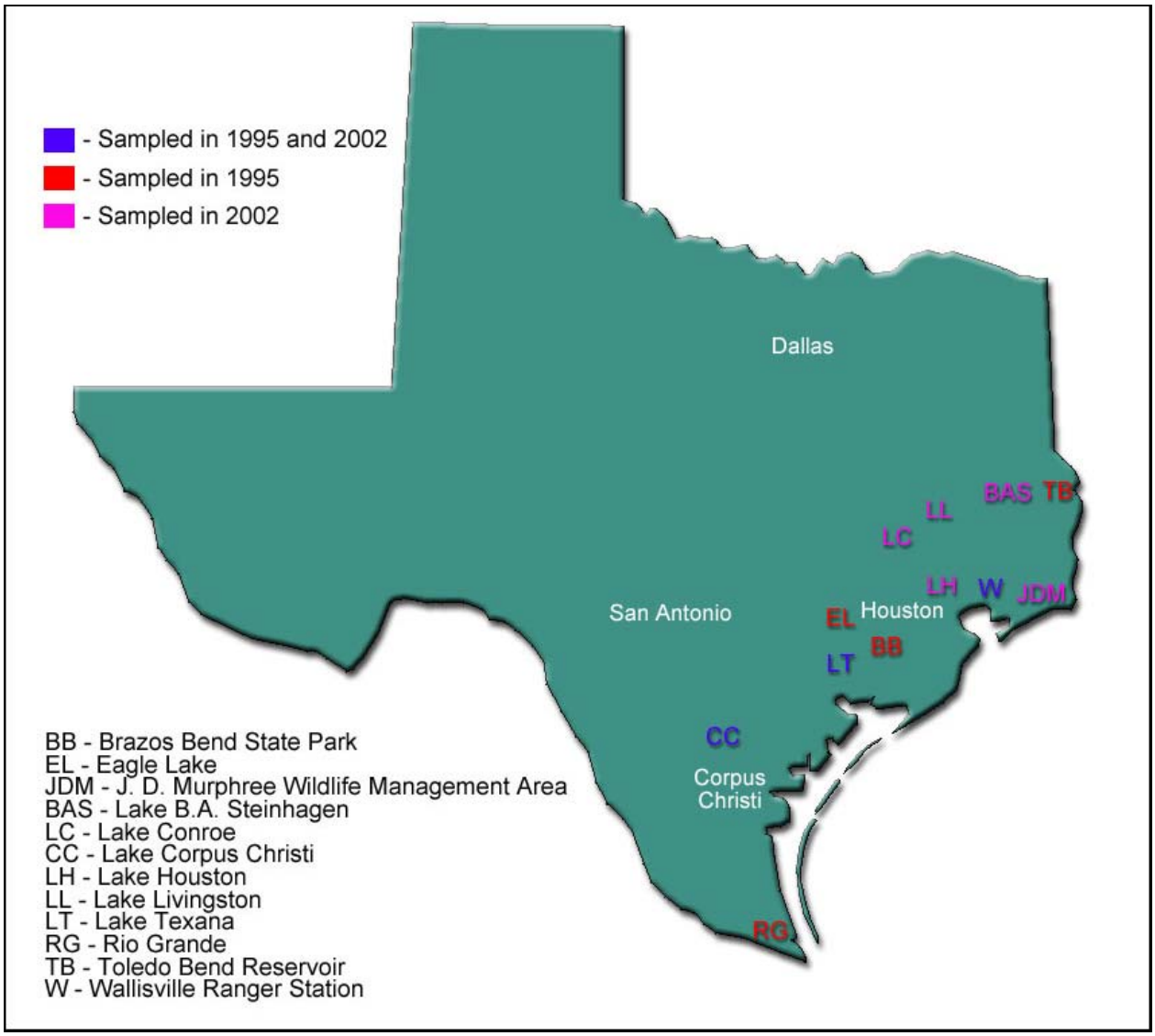

Figure 2. Map of Texas sites sampled in 1995 and 2002

It is important that levels of microsporidia infection remain low in Texas to reduce impacts to *Neochetina* populations. One factor that may significantly influence infection rates is the importation of weevils from other localities in the United States that have higher infection rates. It is becoming increasingly more commonplace to have weevils from locations in Florida and Louisiana shipped into Texas for augmentation of existing Texas populations. Unfortunately, microsporidia detection is a difficult and time-consuming process, since there are typically no external symptoms. In addition, detection requires a high degree of specialized training and equipment. The only way to detect the disease is to have a qualified researcher or technician dissect the weevil and examine the internal organs for signs of spores. It is important to perform such examinations periodically on extant and imported weevils to reduce the risk of spreading the disease from infected individuals. Therefore it may be prudent to form regulations in Texas requiring that qualified personnel, prior to release, screen all *Neochetina* spp. for microsporidia. This should minimize the chance of importing high numbers of infected weevils. A certification program should be initiated that will require all

Neochetina spp. imported into Texas to be certified by a competent technical expert on disease levels before allowing the insects into Texas.

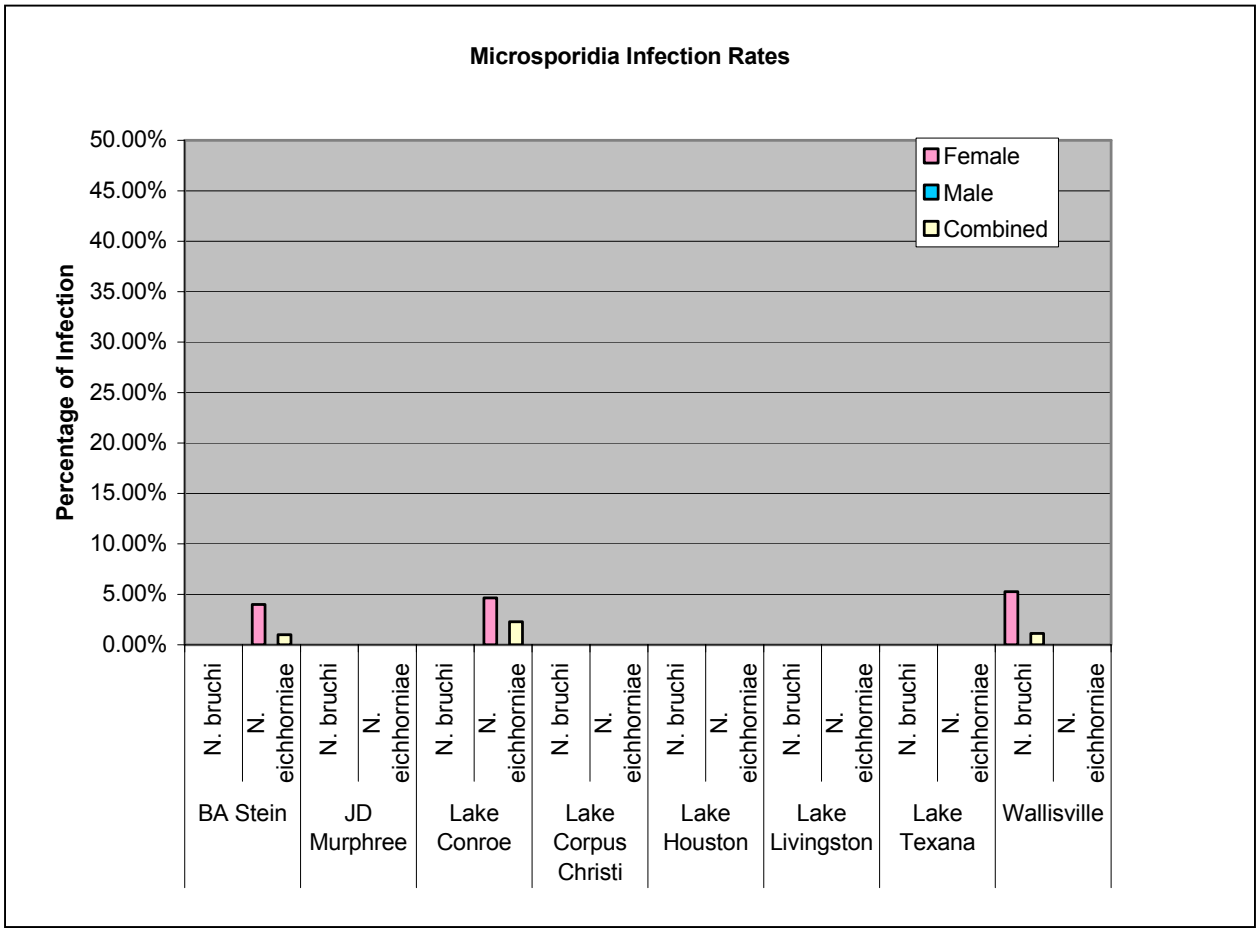

Figure 3. Microsporidia infection rates from the 2002 research in Texas

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