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RESEARCH

Temperature-Dependent Development, Cold Tolerance, and Potential Distribution of *Cricotopus lebetis* (Diptera: Chironomidae), a Tip Miner of *Hydrilla verticillata* (Hydrocharitaceae)

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ABSTRACT. A chironomid midge, *Cricotopus lebetis* (Sublette) (Diptera: Chironomidae), was discovered attacking the apical meristems of *Hydrilla verticillata* (L.f. Royle) in Crystal River, Citrus Co., Florida in 1992. The larvae mine the stems of *H. verticillata* and cause basal branching and stunting of the plant. Temperature-dependent development, cold tolerance, and the potential distribution of the midge were investigated. The results of the temperature-dependent development study showed that optimal temperatures for larval development were between 20 and 30°C, and these data were used to construct a map of the potential number of generations per year of *C. lebetis* in Florida. Data from the cold tolerance study, in conjunction with historical weather data, were used to generate a predicted distribution of *C. lebetis* in the United States. A distribution was also predicted using an ecological niche modeling approach by characterizing the climate at locations where *C. lebetis* is known to occur and then finding other locations with similar climate. The distributions predicted using the two modeling approaches were not significantly different and suggested that much of the southeastern United States was climatically suitable for *C. lebetis*.

Key Words: biological control, distribution, temperature requirement, degree day, predicted distribution

The spatial distribution of species and factors governing their distributions have long been central topics in ecological research (Grinnell 1924, Hutchinson 1957, Guisan and Thuiller 2005). Recent developments in geographic information systems, coupled with the availability of computer software programs and climate databases, have resulted in a proliferation of studies on species distributions (Elith and Leathwick 2009, Franklin 2009). The dominant approach used to model species distributions is to characterize environmental variables at locations where a species is known to occur and then predict the presence of the species in other areas that share a similar environment. This method has been referred by several names but most commonly as “ecological niche modeling” or “species distribution modeling” (Elith and Leathwick 2009, Franklin 2009). The environmental variables most often used to characterize locations where a species is present are climatic, as climate tends to determine distributions of organisms, at least at large spatial scales (Guisan and Thuiller 2005, Ebeling et al. 2008). The second method relies on physiological information about a species tolerance to environmental variables, often gained through experimentation, and then finding locations with environments that fall within those tolerances (Lapointe et al. 2007, Magarey et al. 2007, Diaz et al. 2008). Herein, this approach is termed “physiological modeling,” although it has also been called “ecophysiological modeling” or “mechanistic niche modeling” (Kearney and Porter 2009). Ecological niche modeling has the advantage of not requiring detailed biological information about a species, whereas physiological modeling does not require a priori knowledge of a species’ distribution. Rarely both approaches are used concurrently to predict a species distribution.

Cricotopus lebetis Sublette (Diptera: Chironomidae) is a nonbiting midge that was first discovered in the United States in 1957 in northern Louisiana (Sublette 1964) and later found at other locations in

Louisiana and Florida (Appendix A). The origin of the midge is unknown, but there is speculation that it is an introduced species (Epler et al. 2000, Cuda et al. 2002). As with most chironomids, immature stages are aquatic (Oliver et al. 1990). Little was known about the life history of midge until the early 1990s, when larvae were found boring in the apical meristems of *Hydrilla verticillata* (L.f. Royle) (Hydrocharitaceae) (hereafter hydrilla) in Crystal River, Florida (Cuda et al. 2002). As extensive systematic sampling for the midge has not been conducted, it is unknown how well the occurrence records reflect the actual range.

Hydrilla is a highly aggressive exotic macrophyte that is found throughout Florida and other parts of North America. There have been at least two separate introductions of hydrilla into the United States, with a dioecious form found in the southeast and California, and a monoecious form in the northeast, Atlantic coast, midwest, and California (Madeira et al. 2000). The dioecious form of hydrilla was imported from Sri Lanka into the United States in the late 1950s through the aquarium trade (Schmitz et al. 1991), whereas the monoecious type, which probably originated in Korea (Madeira et al. 1997), was first found in the United States in 1982 near Washington, DC (Steward et al. 1984).

Effective control of hydrilla is difficult to achieve because of a limited number of environmentally sound options (Hoyer et al. 2005). For many years, efforts to control hydrilla relied primarily on the application of the herbicide fluridone. However, fluridone resistance has been documented in several central Florida water bodies, resulting in the use of alternative herbicides such as endothall and acetolactate synthase inhibitors (Netherland et al. 2005, Koschnick et al. 2007). Therefore, new management approaches to control hydrilla populations are being investigated (Cuda and Gillett-Kaufman 2011).

Biological control is one possible management approach, used either alone or integrated with other tactics. Cuda et al. (2011)

demonstrated that in the laboratory *C. lebetis* was able to suppress the growth of hydrilla by >90%, and thus, the midge may have value for biological control. This could be accomplished by augmentation of existing populations in areas where the midge already occurs or introducing the midge into areas where it does not occur. Temperature will influence the midge's population growth, and to a large extent, determine limits to the midge's spatial distribution, but there is no information on the effect of temperature of the life history of *C. lebetis*.

The purpose of this study was to determine the influence of temperature on survival and developmental rate of *C. lebetis*, and to use this information to predict areas conducive to its establishment and population growth. In addition, the midge's potential distribution was predicted using an ecological niche model by extrapolation of the climate at locations where it is known to occur, to other locations where its presence has not been studied. The predicted distributions of *C. lebetis* derived from physiological data and from the ecological niche model were compared to examine the degree of overlap between the two models.

Materials and Methods

Source and Culturing of *H. verticillata* and *C. lebetis*. Hydrilla was collected from Lake Tohopekaliga, Osceola Co., Florida (28.2° N, 81.4° W), and *C. lebetis* was collected from Lake Rowell, Bradford Co., Florida (29.9° N, 82.1° W). Both cultures were maintained at the University of Florida's Biological Control Research and Containment Laboratory, Fort Pierce, FL. Hydrilla was propagated from stems (10–30 cm) collected at the field site and planted in 10.0 by 9.0 cm (diameter by height) pots containing a layer of potting soil (~5 cm) covered by a layer of sand (~2 cm). The pots were placed in a large plastic livestock watering tank (378 liters) filled to a depth of 50 cm with well water and covered with 60% shade cloth to suppress algal growth. Growing tips were harvested as needed.

C. lebetis was reared by placing hydrilla tips in well water in a plastic container (34 by 28 by 15 cm, length by width by height) held inside a cubic cage (50 cm each side) constructed from polyvinyl chloride tubing covered with fine nylon mesh cloth. *C. lebetis* egg masses were added to the containers and larvae colonized the hydrilla tips. Emerging adults were collected using an aspirator and transferred to a 250 ml separatory funnel with ~15 ml of well water. Females oviposited on the water surface and egg masses were collected by opening the stopcock of the separatory funnel (see Cuda et al. 2002 for further details on rearing).

Survival and Developmental Time. Temperature-dependent development of *C. lebetis* was investigated during a single study in environmental chambers maintained at a constant photoperiod (14:10 [L:D], h) and 10 constant temperatures (10, 15, 20, 22, 25, 27, 30, 32, 35, 36 ± 1°C). Healthy, undamaged plant tips, 4–6 cm in length, were placed individually in 35-ml test tubes filled with well water as described by Cuda et al. (2002). Each test tube was placed in a rack that held 40 tubes. Two newly hatched larvae were transferred to each plant tip using a pipette. Once the larvae were introduced into the tubes, a cap with ventilation holes was placed on each tube. One hydrilla tip was typically used, but on a few occasions, destroyed tips were replaced to allow midge larvae to complete development to adulthood. The proportion of individuals surviving at different temperatures was compared with a chi-square test followed by Tukey's procedure for separation of proportions (Elliott and Reisch 2006). Developmental rate at different temperatures was analyzed using linear regression. The linear portion (15–35°C) of the developmental rate curve [$R(T) = a + bT$] was modeled using least squares regression, where T = temperature, a = intercept, and b = slope. The lower temperature threshold for development was estimated as the intersection of the regression line and the x-axis ($R(T) = 0$). Degree-days were calculated as the inverse slope of the fitted regression line (Campbell et al. 1974).

The relationship between developmental rate and temperature was also examined with the nonlinear Brière-1 model, which allows

estimation of upper and lower developmental thresholds (Brière et al. 1999). The model is defined as $R(T) = aT(T - T_0)(T_L - T)^{1/2}$, where R = developmental rate, T = temperature, T_0 = base temperature threshold, T_L = lethal temperature, and a = empirical constant. T_0 and T_L were initially set to 6 and 36°C, respectively, and the equation was then solved iteratively using PROC NONLIN (SAS Institute 2008).

Estimation of Generations per Year. Daily minimum and maximum temperatures from Florida were obtained from 91 weather stations through the Applied Climate Information System (Climate Information for Management and Operational Decisions, Southeast Regional Climate Center; <http://acis.sercc.com>). Daily minimum and maximum temperatures were averaged from the last 5 to 10 yr (1 January 2002 to 1 January 2012) depending the availability of data, which provided 365 values for maximum and minimum temperatures for each station.

The DegDay program version 1.01, which is an Excel (Microsoft Redmond, WA) application developed by University of California-Davis (<http://biomet.ucdavis.edu>), was used to calculate accumulated degree-days for *C. lebetis*. This application uses the upper and lower temperature thresholds for an organism, and daily average of minimum and maximum temperatures, to calculate accumulated degree-days (Baskerville and Emin 1969). The lower and upper temperature thresholds were estimated from the Brière-1 nonlinear model as 9.5 and 36.0°C, respectively. The linear regression model was used to calculate the degree-days (K) required for one generation of *C. lebetis* [$R(T) = abT$] as $K = 1/b$ (Campbell et al. 1974). The prediction of the number of generations per year was calculated by dividing the cumulative degree-days per station by K .

Weather station name, latitude, longitude, and number of *C. lebetis* generations per year were imported into ArcGIS 9.0 (ESRI Inc., Redlands, CA). The imported file was converted to a shape file using the ADD X-Y DATA function followed by the selection of the State Plane Projection. A shapefile of the border of Florida was obtained from the AWhere Continental database (AWHERE, Inc., Denver, CO) and used to delineate the range of predictions.

The geostatistical analysis function in ArcGIS (ESRI Inc.) was used to generate a predicted distribution of *C. lebetis* generations across Florida. Values at unsampled locations were estimated by interpolation of values at sample locations. The inverse-distance-weighted (IDW) deterministic method was used, where predictions are made by mathematical formulas that generate weighted averages of nearby known values. The IDW model gives more influence to points that are closer than to ones that are farther away. The parameters used in the IDW analysis were as follows:

- The number of stations used for interpolation was set to a maximum of 15 and minimum of 10.
- The power optimization option was selected generating a power value of $p = 2$. This weights weather station values proportional to the inverse distance raised to the power p .
- The search neighborhood shape was circular because there were no directional influences on the weighting of number of generations per station. Ellipse parameters were set to: angle, 0 major and minor semiaxis, 1020596.

Cold Tolerance. A cold tolerance study was conducted using second- to fourth-stage larvae. Although there is no information on overwintering of *C. lebetis*, larvae were used for the cold tolerance study, because this is the typical overwintering life stage of chironomids (Pinder 1986, Tokeshi 1995). Four second- to fourth-stage larvae were placed inside a 35-ml vial containing two hydrilla tips and well water. Insects were acclimated from 20°C to the final temperature at intervals of 5°C every 2 h. Larvae were exposed to three constant temperatures (5, 7.5, and 10°C) for 0.5, 1, 2, 4, 8, 16, and 32 d. In total, five vials of four insects were assayed at each time interval at the constant temperatures of 5 and 7.5°C. After each exposure time, insects were placed at room temperature, and survival was assessed by observing for movement once the

water reached room temperature. The effect of exposure times on midge survival was analyzed using logistic regression (PROC LOGISTIC, SAS Institute 2008). The LT₉₀ (lethal times of 90% of tested individuals) at 5 and 7.5°C were used to predict isothermal lines delineating regions in the southeastern United States favorable for *C. lebetis* establishment based on historical weather data. A model was created in the North Carolina State University, Animal Plant Health Inspection Service, Plant Pest Forecasting System (NAPFAST); an internet-based system that allows users to mine weather databases and link this information with templates for biological modeling (Borchert and Magarey 2007, Magarey et al. 2007). Using NAPFAST, the number of days at or below 5 and 7.5°C were recorded at weather stations across the southeastern United States. A probability map was generated using the last 10 yr of weather data to examine the frequency of occurrence of reaching the LT₉₀ in at least 5 out of 10 yr. The map was imported into ArcGIS 9.0 and converted to an ESRI band interleaved by line (bil) raster layer. The area within the continental USA south of the projected LT₉₀ line was extracted from the layer using the “extract” tool in the Spatial Analyst Tools. The raster was then reclassified to make all raster cell values equal to one to provide a continuous grid layer of the predicted area.

Ecological Niche Modeling. Geographic coordinates of locations where *C. lebetis* was collected were obtained from voucher specimens, literature, and known field collection sites, including our own data and data provided by Dana Denson (Reedy Creek Improvement District), Doug Strom (Water and Air Associates, Inc.) and Robert Rutter (Florida Department of Environmental Protection; see Appendix A for list of records). The Maximum Entropy Species Distribution Model (MaxEnt, version 3.3.3e) was used to analyze climate at locations where *C. lebetis* was known to occur and find locations with similar climate to predict the potential distribution of *C. lebetis* (Phillips et al. 2006). MaxEnt estimates the probability of a species presence by contrasting the probability density of environmental variables across known occurrences of the species to that of randomly selected pseudo-absences from the target landscape or model background. Following the method used in Webber et al. (2011), Köppen-Geiger climate zone polygons (Köppen 1936; available from CliMond 10' historical climate data, Kriticos et al. 2011) containing one or more records of *C. lebetis* was used as a background (see Appendix B). The default setting of MaxEnt was used (Mukherjee et al. 2011). Six temperature-related bioclimatic variables (Table 1) with a spatial resolution of 10 arc-second were used to predict *C. lebetis* distribution (Kriticos et al. 2011). Only temperature variables were included in the model because the midge is aquatic in its immature life stages, and therefore, precipitation-related variables were assumed to have minimal direct effect. Air temperature was used rather than water temperature because no large-scale spatial layer of water temperature was available, and water surface temperature tends to be well correlated with air temperature (McCombie 1959). The prediction was limited to the southeastern United States to avoid extrapolation beyond known occurrences. The continuous probability prediction generated by MaxEnt was converted to binary (presence or

absence) prediction using the lowest presence threshold, defined as the minimum nonzero predictive value received by any known occurrence (Pearson et al. 2007).

Comparison of Distributions Predicted from Cold Tolerance Data and from Ecological Niche Modeling. Following Mukherjee et al. (2012), overlap in bioclimatic space predicted by the MaxEnt ecological niche model and the NAPFAST cold tolerance model were examined by principal component analysis (PCA) of a covariance matrix of the six bioclimatic variables. A Monte-Carlo test with 99 repeats ($\alpha = 0.05$) was used to determine the statistical difference between the centroids of the climatic polygons predicted by MaxEnt and the

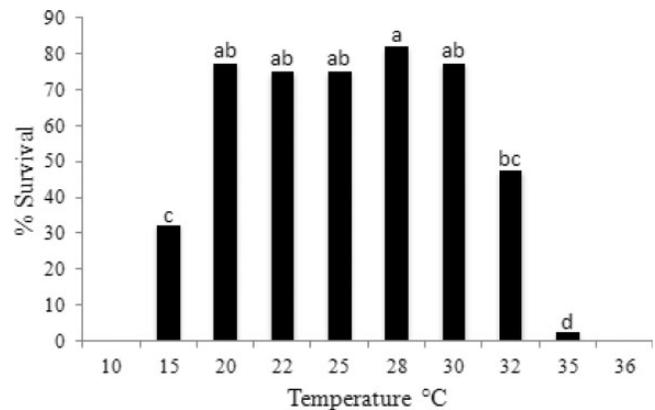


Fig. 1. Percent survival of *C. lebetis* larvae at constant temperatures. Different letters above bars represent statistically different means (analysis of variance and Student-Newman-Keuls (SNK) test, $P < 0.01$). Error bars \pm SEM.

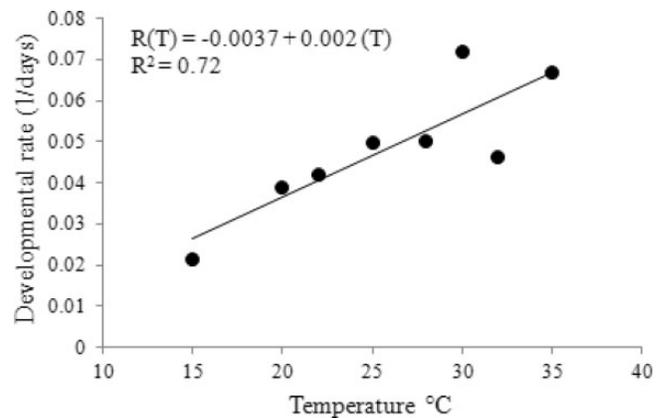


Fig. 2. Developmental rate of *C. lebetis* larvae at constant temperatures between 15 and 35°C.

Table 1. Bioclimatic variables and their loadings on three PCA axes used to examine the similarity between the distributions predicted by the MaxEnt ecological niche model and the NAPFAST physiological model

Climate variables	Variable descriptions	Axis 1 (81%)	Axis 2 (16%)	Axis 3 (2%)
Bio1	Annual mean temperature	0.955	0.283	-0.078
Bio4	Temperature seasonality (SD \times 100)	-0.936	0.322	0.142
Bio6	Min. temperature of coldest month	0.991	-0.066	0.105
Bio7	Temperature annual range	-0.907	0.373	-0.195
Bio10	Mean temperature of warmest quarter	0.510	0.858	0.064
Bio11	Mean temperature of coldest quarter	0.994	-0.003	-0.106

Values > 0 indicate a positive contribution, whereas those < 0 indicate a negative contribution to the axis. Values in parenthesis denote the percent of variability described by each axis, cumulatively explaining $\sim 99\%$ of variability.

NAPFAST models (Mukherjee et al. 2012). Convex hulls were constructed to delimit the climate space predicted under each approach. Climatic variation within a climate space was illustrated with a 1.5 SD inertia ellipse around the centroid of climate space. Correlation circles were constructed to indicate the contribution of each climatic variable to the PCA axes. To examine spatial coincidence between MaxEnt and NAPFAST predictions, centroids of grid cells predicted by each model were extracted. Spatial intersection between model predictions was examined by calculating the percentage of centroid overlap and the percentages of unique centroids for each model.

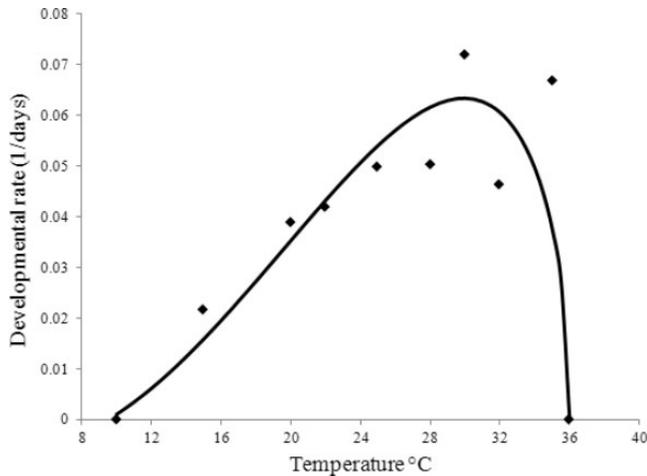


Fig. 3. Brière-1 nonlinear model of the relationship of temperature and developmental rate of *C. lebetis*. The estimated upper and lower thresholds are 36.0 and 9.5°C, respectively.

Results

Survival and Developmental Time. Larval *C. lebetis* survival varied with temperature ($\chi^2 = 95.6$, $P < 0.0001$; Fig. 1). Larvae could not complete development at low and high temperature extremes (10°C and 36°C, respectively). Only a single individual was able to complete development at 35°C. Survival to adulthood was highest at temperatures between 20 and 30°C, and survival rate peaked at 75–80% (Fig. 1). Survival rate was approximately halved at the low and high temperature thresholds of 5 and 32°C, respectively. The development rate increased with increasing temperature, until reaching 32°C ($F_{1,6} = 15.5$, $P < 0.008$; Fig. 2). Degree-day requirements (K) were calculated to be 495.

The Brière-1 model fit the data well with a pseudo $R^2 = 0.76$ (Institute for Digital Research and Education, University of California at Los Angeles [IDRE] 2012). The estimated lower and upper developmental thresholds were 9.5 and 36.0°C, respectively (Fig. 3). These values were very similar to those found in the laboratory tests (Fig. 1). The model showed that the rate of development increased with temperature until the curve reached an optimum of about 30.0°C and then decreased rapidly as the temperature approached the upper developmental threshold (Fig. 3).

Based on degree-day requirements, *C. lebetis* is predicted to complete several generations per year in Florida, ranging from 6.8 to 11.7, with the most generations estimated for the southern portion of the state, and the fewest in the panhandle (Fig. 4). Florida counties located south of Palm Beach County had the highest number of estimated generations ranging from 10.2 to 11.7. Counties in the middle portion of the state are predicted to support 8.2–10.2 generations per year.

Cold Tolerance. After 4 d at 5°C, larvae survival rapidly decreased (max-rescaled $R^2 = 0.76$; Fig. 5A). Only 50 and 10% of larvae were able to survive for 8 and 16 d, respectively. No insects were able to survive after 32-d exposure to 5°C (Fig. 5A). After 16 d at 7.5°C, the

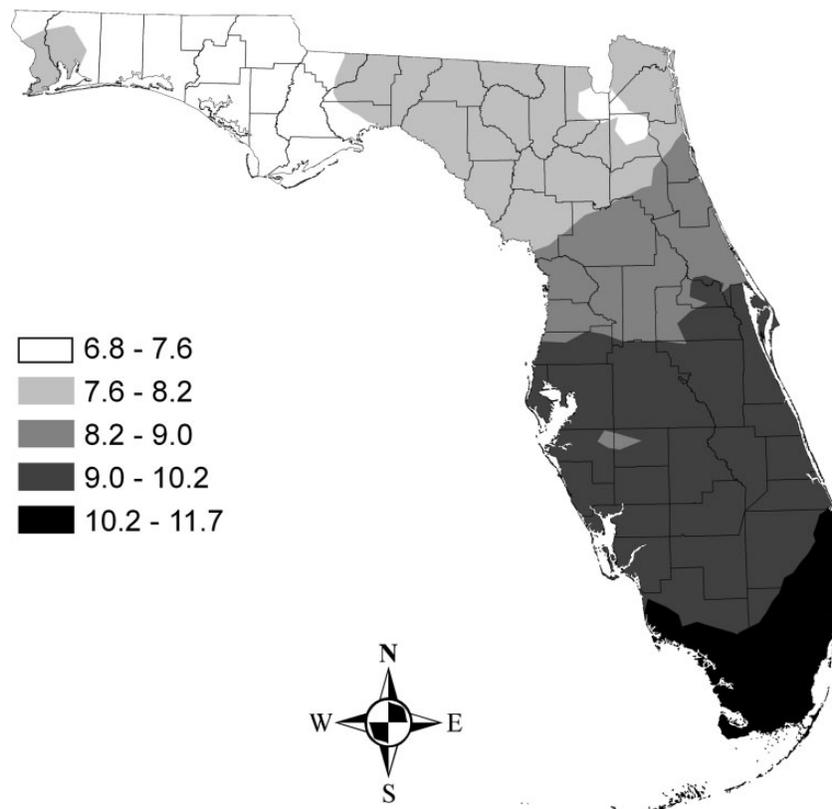


Fig. 4. Predicted number of generations per year of *C. lebetis* in Florida.

survival of larvae remained close to 100% (max-scaled $R^2 = 0.88$; Fig. 5B). The isothermal lines showed that at both 5 and 7.5°C, 90% mortality of *C. lebetis* was predicted to occur along a line running from southeastern South Carolina, extending through the middle of Georgia, Alabama and Mississippi, northern Louisiana, and through the mid-section of Texas (Fig. 6). Similar to the NAPPFAST model, MaxEnt predicted the distribution of *C. lebetis* in the much of southeastern United States (Fig. 6).

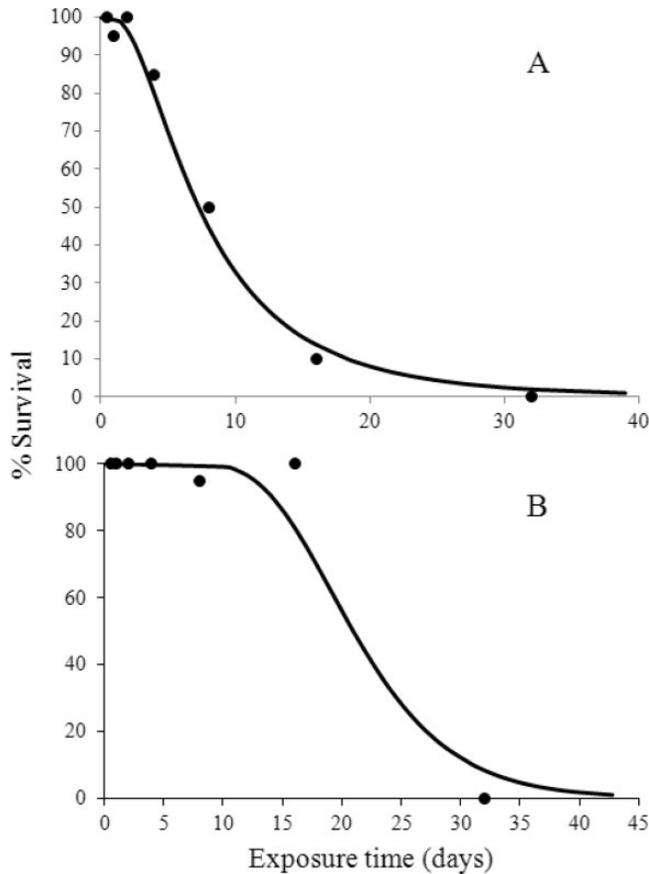


Fig. 5. Survival of *C. lebetis* larvae at different exposure times at (A) 5°C and (B) 7.5°C. Single dots are observed values, and lines are expected value of the logistic regression. Maximum survival occurred at 7.5°C with an exposure time of 16 d.

Comparison of Distributions Predicted From Cold Tolerance Data and From Ecological Niche Modeling. PCA resulted in three significant axes explaining 99% of variability (Table 1). Graphical representation of climate space across first two components showed no significant difference in climate space predicted by the MaxEnt and NAPPFAST models (Fig. 7). As evident by the extensive overlap of predicted climate spaces, no difference between the climate spaces was found by the between-class analysis of variance using 99 Monte-Carlo randomizations (between-class inertia percentage = 0.09, $P = 0.12$). Of all the grid centroids, 81.4% were predicted by both MaxEnt and NAPPFAST models (Fig. 6). The MaxEnt model predicted 16.9% unique centroids and the NAPPFAST model 1.7%.

Discussion

Temperature is a critical factor that influences insect distributions (Andrewartha and Birch 1954, Munroe 1984). Understanding the thermal requirements for *C. lebetis* is an important step for predicting the midge's distribution. Water temperatures during the winter months throughout Florida range from 8 to 15°C (Beaver et al. 1981), and these temperatures are mostly above the lower developmental threshold of 9.5°C. The predicted number of generations per year of *C. lebetis* increased as latitude decreased, suggesting that the midge may reach higher densities in south Florida than further north. However, in summer months, maximum water temperatures in Florida lakes range as high as 30–35°C (Beaver et al. 1981), which approaches the estimated upper developmental threshold of 36°C, and includes the temperature at which the midge's development rate began to slow (32°C). Water temperatures in hydrilla mats near the water surface tend to be higher than water temperatures at a 1-m depth in hydrilla stands or at the surface in open water (Bowes et al. 1979, Cuda et al. 2008). Temperatures reaching 45°C in vegetation mats have been reported (Wheeler and Center 2001), which is well above the upper lethal threshold of *C. lebetis*. Wheeler and Center (2001) partially attributed the poor performance of the introduced biological control agent of hydrilla, *Hydrellia pakistanae* Deonier (Diptera: Ephydriidae), to high temperatures in hydrilla mats. Likewise, *C. lebetis* is likely to suffer high mortality during summer months, particularly in south Florida, resulting in possible local extirpation. Although there is no information available on the dispersal ability of *C. lebetis*, in general chironomids are considered to be weak flyers (Delettre and Morvan 2000). Thus, high temperatures could play a critical role in determining the performance and persistence of *C. lebetis* in Florida and other areas where water temperatures approach or exceed the upper lethal threshold.

The physiological model based on cold tolerance of midge larvae predicted survival of the midge in much of the southeastern United States. The area south of the LT_{90} isothermal line includes all of Florida, about one-half of Georgia, Alabama and Mississippi, most of Louisiana, and the southern half of Texas. A reasonable prediction for

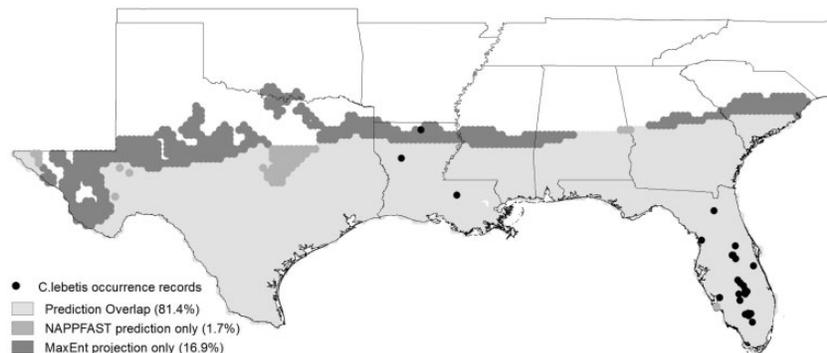


Fig. 6. Predicted distributions of *C. lebetis* based on the NAPPFAST model using the area south of the LT_{90} isothermal line for survival of larvae at 5°C and the predicted distribution based on the MaxEnt ecological niche model using six climate variables (see text). Values in parentheses following the legends for the NAPPFAST and MaxEnt predictions indicate percent of unique grid cells predicted by each method.

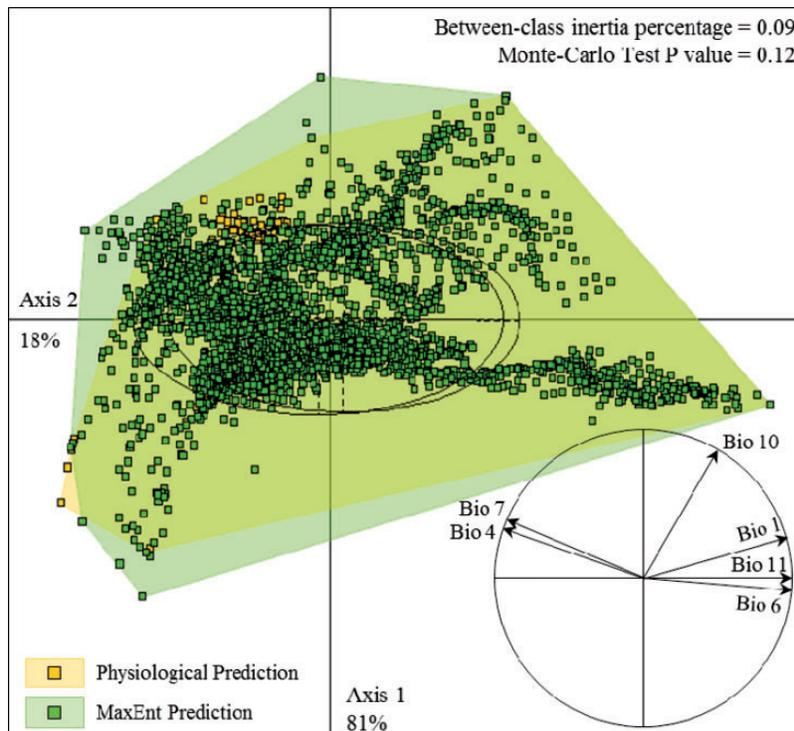


Fig. 7. Model prediction of climate suitability for *C. lebetis* using known sampling locations and climate records. Inertia ellipses (1.5 SD) were drawn around the centroids of climatic envelopes predicted by the physiological and MaxEnt models. The enclosed correlation circle describes the importance of individual bioclimatic variables along the two PCA axes. See Table 1 for descriptions of climatic variables and contribution of individual variables to PCA axes. Note that some physiological prediction symbols (yellow squares) are hidden beneath the MaxEnt symbols.

areas that would support positive population growth of *C. lebetis* would be below the LT_{90} at 5°C. Lapointe et al. (2007) used a combination of LT_{50} and LT_{95} values, along with field observations, to predict areas suitable for the establishment of the introduced root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) in Texas, Arizona, and California, and Diaz et al. (2008) speculated that the introduced biological control agent, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), would establish south of LT_{50} or LT_{90} isothermal lines. Our prediction for *C. lebetis* is based on the assumption that temperature alone influences the midge's distribution, which may be reasonable on a coarse (country wide) spatial scale. On a finer scale, the midge can only occur in aquatic environments, and host plants must be present. Host plant availability is probably not highly limiting because at least one of the midge's host plants, hydrilla, is found in water bodies throughout the southern United States (Madeira et al. 2000), and *C. lebetis* has been shown to complete development in several other common aquatic plants (Stratman et al. 2013).

The MaxEnt ecological niche model, based on six temperature variables, also predicted that suitable locations for the establishment of *C. lebetis* are present throughout the southeastern United States, but the prediction extended further north than that of the NAPPFAST model. The difference in the outputs from the two modeling approaches may be due to the origin of the insects used in the cold tolerance study. The midge colony was initiated with individuals collected from Lake Rowell in Bradford Co., Florida (29.92° N, 82.16° W), which is considerably south of the most northern known occurrence of *C. lebetis* in Farmersville, Louisiana (32.75° N, 92.4° W; Sublette 1964). Intraspecific variation in cold tolerance among insects is a common occurrence (Turnock and Fields 2005), and thus, it would not be surprising if our colony had a lower tolerance for cold than populations occurring further north.

Very few studies have incorporated both niche and physiological models to predict potential distribution of a species. Ebeling et al.

(2008) predicted the distribution of the invasive ornamental plant, *Buddleja davidii* F. (Lamiales: Scrophulariaceae), by combining physiological data on frost tolerance with an ecological niche model. Elith et al. (2010) incorporated information from MaxEnt and a physiological model to predict the distribution of the invasive cane toad [*Bufo marinus* (L.) (Anura: Bufonidae)] in Australia. In both cases, the authors concluded that incorporating information from physiological models can improve the reliability of distributional predictions. Although the MaxEnt model in this study yielded a slightly larger geographic prediction than the physiological model, there was no statistical difference in the distributions predicted by the two modeling methods. The spatial coincidence of these two approaches increases confidence in the prediction. However, the MaxEnt model should be interpreted with caution as the presence data were limited to 3 records from Louisiana and 25 from Florida. Because the native range of this insect is unknown, it could possibly be narrowed down by identifying areas with climate similar to that found in the southeastern United States.

Temperature-dependent development and cold tolerance studies provide basic information that can be used to develop or improve rearing methods, and to predict field colonization and establishment. Temperature studies revealed that optimal temperature conditions for *C. lebetis* are from 20 to 30°C, with lower survival occurring at 15 and 32°C. This could explain why this insect is widely established in Florida and also occurs in Louisiana. Ongoing studies on population dynamics and field impact will reveal the potential value of *C. lebetis* as an augmentative biological control agent.

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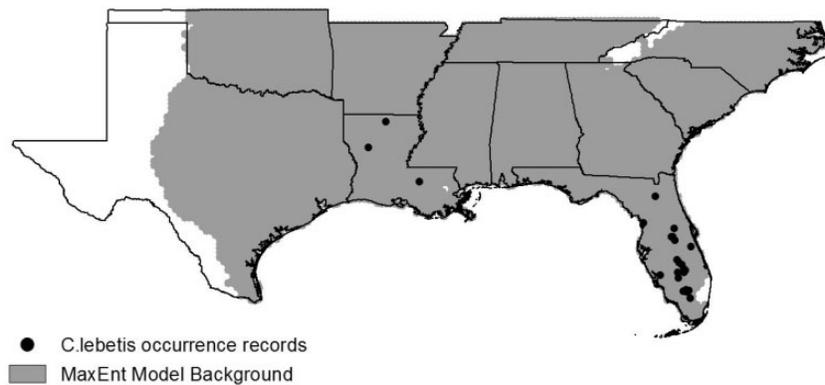
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Appendix A. North American occurrence records of *C. lebetis*^a

Location	Latitude	Longitude	Source
Baton Rouge, LA	30.4619° N	91.1431° W	Epler et al. (2000)
Natchitoches, LA	31.7610° N	93.0861° W	Sublette (1964)
Farmersville, LA	32.7528° N	92.4046° W	Sublette (1964)
Lake Tohopekaliga, FL	28.2414° N	81.4064° W	K.N.S. (unpublished data)
Lake Istokpoga, FL	27.3944° N	81.2445° W	K.N.S. (unpublished data)
Bulldozer Canal, FL	27.9976° N	80.7939° W	K.N.S. (unpublished data)
Lake Rowell, FL	29.9185° N	82.1603° W	K.N.S. (unpublished data)
Crystal River	28.8917° N	82.6033° W	Cuda et al. (2002)
Lake Brantley, FL	28.6935° N	81.4210° W	D. Denson (personal communication)
Village Lake, FL	28.3702° N	81.5325° W	D. Denson (personal communication)
EPCOT Lake, FL	28.3678° N	81.4956° W	D. Denson (personal communication)
West Feeder Canal, FL	26.3012° N	81.0728° W	D. Strom (personal communication)
West Feeder Canal, FL	26.2907° N	80.9720° W	D. Strom (personal communication)
North Feeder Canal, FL	26.3390° N	80.9796° W	D. Strom (personal communication)
North Feeder Canal, FL	26.2913° N	80.9703° W	D. Strom (personal communication)
L4 Canal, FL	26.3299° N	80.8826° W	D. Strom (personal communication)
28 Interceptor Canal, FL	26.2586° N	80.9532° W	D. Strom (personal communication)
L28 Borrow Canal, FL	26.3299° N	80.8807° W	D. Strom (personal communication)
L28 Borrow Canal, FL	26.0278° N	80.8303° W	D. Strom (personal communication)
Harney Pond Canal, FL	27.0911° N	81.0702° W	D. Strom (personal communication)
Harney Pond Canal, FL	27.0164° N	81.0702° W	D. Strom (personal communication)
Indian Prairie Canal, FL	27.1526° N	81.0685° W	D. Strom (personal communication)
Indian Prairie Canal, FL	27.0977° N	81.0104° W	D. Strom (personal communication)
South Lake Istokpoga, FL	27.3039° N	81.1299° W	R. Rutter (personal communication)
Caloosahatchee River, FL	26.7868° N	81.2655° W	R. Rutter (personal communication)
Arbuckle Creek, FL	27.5025° N	81.3354° W	R. Rutter (personal communication)
North Prong Alligator Creek, FL	26.8952° N	81.9702° W	R. Rutter (personal communication)
Southwest, FL	27.0200° N	81.3026° W	R. Rutter (personal communication)

^aGeopositions of records provided by Dana Denson, Doug Strom, and Robert Rutter are estimated from label descriptions and personal communications.



Appendix B. MaxEnt model background used to predict potential distribution of *C. lebetis*. Model background is based on the Köppen–Geiger climate zone polygons containing one or more records of *C. lebetis*.