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Regional-scale climate-induced variation in ant physiology and morphology

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Regional-scale climate-induced variation in ant physiology and morphology

by

Daniel Egan Murphy

An Abstract for a Thesis in Biology

Submitted in Partial Fulfillment

of the Requirements

for the Degree of

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Buffalo State College

State University of New York

Department of Biology

ABSTRACT OF THESIS

The biology of ectotherms such as insects is influenced by ambient thermal conditions. Ants are a ubiquitous and ecologically important group of insects and are well-established as bioindicators of thermal conditions. Ants are sensitive to the thermal extremes that vary with latitude, elevation, and land use, and these thermal gradients influence their spatial and temporal distributions. As a result, ants have evolved physiological and morphological thermal adaptations in response to the thermal environment of their habitats. These adaptations include increased physiological and morphological tolerance for temperature extremes. In Western New York (WNY), temperatures are temporally and spatially heterogeneous, changing with the season and in distinct regional climate zones formed by gradients in elevation and proximity to the Great Lakes. Coastal areas are relatively colder in spring and warmer in fall than inland areas, and urbanized areas are relatively warmer year-round. The goal of this study is to assess ants as climate bioindicators by investigating the relationship between ant thermal traits and regional climate variation in Western New York. Though I investigated several ant species, my focus was on two regionally abundant ant species: *Aphaenogaster picea* (myrmicine) and *Lasius americanus* (formicine). I repeatedly sampled closed canopy and open areas during the early and late summer of 2020. Ants were tested for minimum and maximum physiological thermal tolerance (CT_{\min} and CT_{\max}) and ant leg length morphology was measured using a stereomicroscope. I found no relationship between variation in *A. picea* or *L. americanus* physiological and morphological thermal traits and variation in WNY climate zones. CT_{\min} decreased throughout the summer season across all species irrespective of climate zone, and CT_{\max} increased across all species throughout the summer season in the Niagara Frontier and

Southern Tier zones, but decreased in the Erie, Ontario Coastal, and Urban zones. Species-specific effects were present, with CT_{max} in *A. picea* increasing across the summer season in the Niagara Frontier, Southern Tier, and Erie Coastal zones while decreasing in the Ontario Coastal and Urban zones. Femur length increased slightly across all species throughout the summer season irrespective of climate zone, with the greatest increase seen in *A. picea*. Femur length did not increase in *L. americanus*. Overall, these results suggest that there is no fine-scale influence of climate zone on ant thermal traits, but the traits are not static as they varied with season. Whereas morphological traits showed slight variation with season, physiological traits varied relatively more with season.

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Introduction

Ectotherms are animals with metabolism dependent upon the external environment (Buckley et al. 2012). They are unable to regulate body temperature internally and must either conform to or mitigate ambient thermal conditions (Hoffmann et al. 2013). Insects are globally ubiquitous ectotherms that exhibit physiological and morphological adaptations to function within their specific thermal climates (Addo-Bediako et al. 2000, Deutsch et al. 2008, Lee and Denlinger 2010). For example, insects in extreme cold environments can survive by entering a reversible state of metabolic arrest, whereas some desert-dwelling insects, such as ants, mitigate heat extremes using reflective hairs (MacMillan and Sinclair 2011, Willot et al. 2016).

Ants are one of the most geographically widespread insect groups, common within all but a few terrestrial ecosystems, including disturbed and urban habitats (Barden and Grimaldi 2016, Lach et al. 2009, Heuss et al. 2019, Schultz 2000, Santos 2016). In addition to being abundant and widespread, ants also are very taxonomically diverse with over 13,000 species worldwide (Bolton 2020). Because ants are ectothermic, they cope with thermal variation across their terrestrial environments through physiological and morphological adaptations (Diamond et al. 2012). Whereas the great diversity of ants leads to species-level differences in thermal traits there are some similar adaptations across species including shifts in physiological thermal tolerance and morphological femur lengthening (Lach et al. 2009, Sommer and Wehner 2012).

Ants physiologically mitigate adverse heat like most other animals by producing heat shock proteins (HSPs) that function to safeguard cells from stressors including excess heat (Alard et al. 2014, King and MacRae 2015). Ants mitigate extreme cold via metabolic arrest (aka “chill-coma”) (Roeder et al. 2021) through the maintenance of ionic balance between muscles and peripheral nerves (MacMillan and Sinclair 2011). It is thought that ant responses to extreme

heat and cold are controlled by different sets of genes, and these genes may vary between groups of species (Roeder et al. 2021, Nguyen et al. 2016). Moreover, the physiological mechanisms responsible for tolerance to heat and cold in ants are not fully understood (Helms Cahan et al. 2017).

Ants also mitigate ambient climate conditions through morphological adaptations (Willot et al. 2016). Femur length (the length of the third leg segment) corresponds with longer legs, that reduce thermal conduction between the ant body and the ground by raising the ant further upward (Sommer and Wehner 2012). Longer femur length also increases stride, which increases the ant foraging speed which, in turn, reduces the duration of exposure to adverse heat (Sommer and Wehner 2012). In addition, there is a correlation between femur length and overall body size, which itself is a thermal trait in ants because larger insects have a lower surface-to-volume ratio and thus maintain their body temperatures more easily (Verble-Pearson et al. 2014, Stevenson 2015).

Physiological thermal tolerance shapes ant distributions and activities at broad and local scales (Addo-Bediako et al. 2000, Arnan et al. 2015). At broad scales such as across an elevational gradient, cold tolerant ants occupy cooler climates at higher elevations where they are protected from better competing heat tolerator ant species that reside at lower elevations (Warren and Chick 2013, Bishop et al. 2017). At local scales, physiological thermal tolerance influences the spatial distributions and temporal activity of ants given that ambient temperatures dictate their foraging patterns and activity (Arnan et al. 2015, Roeder et al. 2018). For example, ant communities in the forests of Vermont changed under conditions of experimental shading such that foragers of cold tolerator species were more abundant (Wittman et al. 2010).

Whereas ant species vary in their thermal tolerances, which determines broad-scale differences in distributions, species-level plasticity in thermal traits, or variation in how thermal traits are expressed as influenced by environmental cues, is potentially adaptive for some ant species (Rodrigues and Beldade 2020, Bacigalupe et al. 2018). Thermal traits can be static, as with the ant *Tetramorium curvispinosus*, where heritable genetic changes were implicated in causing regional shifts in physiological thermal tolerance across an urban to rural gradient (Martin et al. 2019). *Myrmica* ant species in Western Russia are also seasonally static in their physiological thermal tolerance (Maysov and Kipyatkov 2009). Whereas thermal tolerance traits are static for some species, conditions during development can influence the expression of thermal traits in some insects, thus leading to plasticity (Kellermann and Sgrò 2018). Experimental warming and cooling during brood development can alter physiological thermal traits in ants of *Linepithema humile* (Jumbam et al. 2008, Chown et al. 2009), and both physiological and morphological thermal traits (via body size) in *Aphaenogaster senilis* ants (Oms et al. 2017). Whereas these changes were induced during brood development, workers of multiple ant species in Oklahoma are seasonally plastic in their thermal tolerances as adults under natural conditions (Bujan et al. 2020).

Ants occupy thermally determined microhabitats, which may make them bioindicators across species (Gerlach et al. 2013). However, plasticity in thermal traits may affect the viability of ants as bioindicators of climate (Gerlach et al. 2013). A moderate amount of thermal plasticity in ant species could support their use as bioindicators by making them more stable in the presence of moderate climatic instability (Carignan and Villard 2002). However, extreme thermal plasticity would make them too resilient to climatic changes to elicit a measurable response (Carignan and Villard 2002). Investigating the thermal tolerance limits of ant species

and the degree to which they are plastic would help us to predict how ant species might respond to climatic variation, and thus interpret how shifts in habitat occupation relate to shifts in climate (Israel Del Toro et al. 2015).

Because temperature is a core component of ant microhabitat determination, the richness of ant species assemblages and abundance of ant species can offer insight into how climatic changes impact ant communities on a relatively fine spatial scale (Andersen 1997, Ellison 2012, Diamond et al. 2016). Species richness and composition of ant assemblages has been used to indicate changes in microclimate, as with the decline of the *Tsuga canadensis* (eastern hemlock) tree, where changes in forest canopy cover altered the relative abundance of ant species within the forest by transforming microclimatic conditions (Sackett et al. 2011). Ants have been used as bioindicators of climatic change on a regional scale as well, with the increasing abundance of relatively cold intolerant *Formica exsecta* used to indicate warming in the Russian Far East (Alfimov et al. 2011).

In WNY, regional-scale climate varies with elevation, urbanization, and distance from two Great Lakes, Erie and Ontario. The combined effect of these forces creates five distinct WNY climate zones (Vermette 2020). The WNY coastal areas typically are colder than adjacent inland zones in the early spring but shift to be relatively warmer than the interior by fall. The shoreline of Lake Erie is thermally distinct from the more inland Southern Tier, which is separated by a ridge, and becomes increasingly cooler with rising elevation. Moving southward from Lake Ontario, The Niagara Frontier rises above a glacial escarpment and separates the Ontario shoreline from a highly urbanized zone, the Buffalo metropolitan area. The Buffalo metropolitan area creates an “urban heat island,” urban warming due to decreased albedo and vegetative cover (Tzavali et al. 2015). The Niagara Peninsula acts as a rain shadow, considerably

reducing the amount of precipitation received in the more northern reaches of the region so that warmer areas along the Niagara Frontier are appreciably drier.

The goal of this study was to compare the spatial distribution of ant species, and physiological and morphological adaptations across the WNY climate zones. Given that coastal areas are colder in spring, which is a crucial time for ant temperature-dependent brood development, I predicted that ants in the coastal climate zones would have higher cold tolerance than ants in inland areas (Warren II et al. 2018). However, temperatures between coastal and inland areas invert between spring and fall. If ants were able to seasonally acclimate, I predicted that the physiological thermal tolerance of ants would shift throughout the season, such that coastal ants would become more heat tolerant and inland ants will become more cold tolerant. Given that urban ants are exposed to greater maximum temperatures during daytime and lesser minimum temperatures during nighttime (Tzavali et al. 2015), I predicted that urban dwelling ants would have a higher heat tolerance and narrower range of overall thermal tolerance than rural ants. This prediction is consistent with the urban heat island effect, wherein urban ant populations will often demonstrate higher tolerance for extreme heat and a narrower range of adaptability (Angilletta et al. 2007, Martin et al. 2019). Given that elongated femurs are an adaptation to adverse heat, I also predicted that they would have longer femur lengths than ants sampled from suburban and rural climate zones.

Methods

Study Species

I focused on two target species, one each from two of the major ant subfamilies, *Aphaenogaster picea* (Myrmicinae) and *Lasius americanus* (Formicinae). Both species are broadly distributed

and common throughout WNY (Ellisson et al. 2012, Schär et al. 2018). Whereas the range of *A. picea* is constrained to the eastern half of the United States and Canada, the range of *L. americanus* extends throughout the continental United States and into Canada and Mexico (Guénard et al. 2017). Morphologically, *A. picea* is both larger and longer legged than *L. americanus* (Ellison et al. 2012). Both species forage above ground and are primarily diurnal; hence, sampling occurred during the day (Ivanov 2019, Lubertazzi 2012). As both target species are forest habitat dwelling (Toro et al. 2013) effort was also made to sample in open habitats when possible. However, species composition was not consistent across open habitats and no candidate target species could be identified for these environments.

Study sites

Sampling was split between closed canopy forest and open canopy sites across WNY's five climate zones. Forest sites were selected from wooded areas with closed canopy cover and ample interior space. Open sites consisted of grassy or bare areas with little to no canopy cover. Study sites were located primarily in public parks and state forests, and one study site was located on private property with the owner's consent. The study sites were selected to be stratified between the WNY climate zones with at least 4 sites in each climate zone.

Ant sampling

I collected ants at 58 sites within the WNY climate zones (2-3 sites per day) between June 30, 2020 and August 9, 2020. The sites were sampled in random order to avoid the influence of spatial and temporal autocorrelation within climate zones. At each sampling site, I searched open and closed habitats haphazardly for up to an hour. Upon discovering an ant colony, I collected 15

worker ants using an aspirator. The ants were transferred into 16 mm test tubes containing nest soil (to reduce stress) and plugged with moist cotton (to prevent desiccation). The test tubes were placed in racks within a cooler containing ice with an insulator between the ice and test tube racks. This protocol prevented overheating and ensured safe transfer of subjects back to the lab. Once back at the lab, the ants were immediately tested for physiological cold and heat tolerance.

For the second round of ant sampling (August 18, 2020, to September 12, 2020), I re-sampled 26 of the first-round sites with at least 3 sites in each climate zone. The sites were selected based on the presence of target species and greater abundance of ant nests. The sites were also selected in an effort to sample along a gradient from the lake shore to inland, and along a gradient from the city center of Buffalo to rural areas. No site was resampled until at least a month had passed from the first sampling.

Physiological Assays

Critical thermal tolerance is defined as the thermal minimum (CT_{min}) and maximum (CT_{max}) temperatures at which ants become incapacitated, or incapable of staying upright. This approach is an accepted method to test for physiological thermal tolerance (Overgaard et al. 2012). Five individuals from each sampling site were randomly selected for one of three treatments: CT_{min} , CT_{max} , or control. All of the ants were placed in 16mm glass vials plugged with moistened cotton. For CT_{min} and CT_{max} , the ants were placed in a Thermo Fischer ARCTIC A40 refrigerated water bath (NesLab, ThermoScientific, Portsmouth, NH, USA). For CT_{min} , ants were acclimated at 20°C for 10 minutes before reducing the temperature 1°C per minute. Ants were assessed after each temperature decrease for loss of motor control. An average CT_{min} ($n = 5$) was

determined for each site. For CT_{max} the same procedure was used except that water temperature was increased 1°C per minute from 30°C. Control ants received no temperature treatments but also were monitored for physiological response. After the physiological assays each specimen was preserved in alcohol and subsequently mounted to characterize morphological traits.

Morphological Assay

Posterior femur length, or the length of the third leg segment, was digitally measured using a Leica M125 stereomicroscope with DFC295 digital camera and using the Leica Application Suite software.

Data Analysis

I analyzed CT_{min} , CT_{max} and femur length as functions of date, ant species and climate zone using analysis of variance (ANOVA) models using the R statistical package (R Development Core Team Version 3.5.1 2020). I also added date x climate zone and date x species interaction terms, but these were removed if not significant. *Post hoc* comparisons were made using Tukey's honest significant difference tests.

Aphaenogaster picea and *Lasius americanus* CT_{min} , CT_{max} and femur length were analyzed individually for each species as functions of date and climate zone using ANOVA models with date x climate zone interaction terms.

Results

All ant species

CT_{min} decreased with the summer season for all ants (Fig. 1a; $df = 1$, $SS = 54.62$, $MS = 54.62$; f -value = 34.750, p -value < 0.001; slope = -0.027, $r^2 = 16.0$). Ant CT_{min} differed by species only in that *Camponotus nearcticus* exhibited the lowest minimum temperature tolerance of all the species (Fig. 1b; $df = 6$, $SS = 46.35$, $MS = 7.73$; f -value = 4.915, p -value < 0.001). Ant CT_{min} did not vary across the climate zones ($df = 4$, $SS = 8.40$, $MS = 2.10$; f -value = 1.366, p -value = 0.259). Neither the date x climate zone or species x climate zone interaction terms predicted variation in CT_{min}, and they were dropped from the model.

A date x climate zone interaction effect indicated that CT_{max} increased with the summer season in the Niagara Frontier and Southern Tier zones, and it decreased with summer season in the Erie and Ontario Coastal zones and the Urban zone (Fig. 2a; $df = 4$, $SS = 27.57$, $MS = 6.89$; f -value = 3.201, p -value = 0.015). *Camponotus nearcticus* and 'other' ants had the highest CT_{max} among the ant species whereas *Lasius* species other than *L. americanus* had the lowest (Fig. 2b; $df = 6$, $SS = 188.24$, $MS = 31.37$; f -value = 14.572, p -value = <0.001). The species x climate zone interaction term did not predict variation in CT_{max} and was dropped from the model.

Femur length increased slightly across ant species with summer season (Fig. 3a; $df = 1$, $SS = 0.18$, $MS = 0.18$; f -value = 9.455, p -value = 0.002; slope = -0.001, $r^2 = 0.3$). Femur length was much greater in *Aphaenogaster picea* and the other *Aphaenogaster* species than all the other ants (Fig. 3b; $df = 6$, $SS = 13.97$, $MS = 2.232$; f -value = 121.942, p -value < 0.0001). Femur length was somewhat shorter in the Urban than Southern Tier zone ($df = 4$, $SS = 0.16$, $MS = 0.04$; f -value = 2.183, p -value = 0.074), but overall there was little difference in femur length between climate

zones. Neither the date x climate zone or species x climate zone interaction terms predicted variation in femur length, and they were dropped from the model.

Aphaenogaster picea

Aphaenogaster picea CT_{\min} decreased with the summer season (Fig. 4; $df = 1$, $SS = 11.71$, $MS = 11.71$; $f\text{-value} = 11.649$, $p\text{-value} = 0.001$). There was no effect of climate zone ($df = 4$, $SS = 1.39$, $MS = 0.34$; $f\text{-value} = 0.346$, $p\text{-value} = 0.845$) on CT_{\min} , and the date x climate zone interaction term did not predict variation in CT_{\min} and was dropped from the model. A date x climate zone interaction term indicated that *A. picea* CT_{\max} increased with the summer season in the Niagara Frontier, Southern Tier and Erie Coastal climate zones, and it decreased in the Ontario Coastal and Urban zones (Fig. 5; $df = 4$, $SS = 10.40$, $MS = 2.59$; $f\text{-value} = 2.511$, $p\text{-value} = 0.045$).

Aphaenogaster picea femur length increased with the summer season (Fig. 6; $df = 1$, $SS = 0.05$, $MS = 0.05$; $f\text{-value} = 4.964$, $p\text{-value} = 0.030$). There was no effect of climate zone ($df = 4$, $SS = 0.01$, $MS = 0.01$; $f\text{-value} = 0.336$, $p\text{-value} = 0.852$) on femur length, and the date x climate zone interaction term did not predict variation in femur length and was dropped from the model.

Lasius americanus

Lasius americanus CT_{\min} decreased with the summer season (Fig. 7; $df = 1$, $SS = 15.35$, $MS = 15.34$; $f\text{-value} = 10.570$, $p\text{-value} = 0.002$). There was no effect of climate zone ($df = 4$, $SS = 9.92$, $MS = 2.47$; $f\text{-value} = 1.707$, $p\text{-value} = 0.175$) on CT_{\min} , and the date x climate zone interaction term did not predict variation in CT_{\min} and was dropped from the model. *Lasius americanus* CT_{\max} was not affected by date ($df = 1$, $SS = 4.95$, $MS = 4.95$; $f\text{-value} = 2.313$, $p\text{-value} = 0.139$) or climate zone ($df = 4$, $SS = 4.42$, $MS = 1.10$; $f\text{-value} = 0.517$, $p\text{-value} = 0.724$). *Lasius*

americanus femur length also was not affected by date ($df = 1$, $SS = 0.01$, $MS = 0.01$; f -value = 1.028, p -value = 0.319) or climate zone ($df = 4$, $SS = 0.01$, $MS = 0.01$; f -value = 0.247, p -value = 0.909).

Discussion

Ant species in WNY exhibited far greater temporal than spatial plasticity. As such, climate zone was not predictive of thermal tolerance, making the ants poor spatial climate bioindicators. Moreover, physiological temperature tolerance appeared to be more plastic across the season than morphological femur length.

Ants increased their physiological tolerance for heat as the season progressed in the Niagara Frontier and Southern Tier climate zones and decreased their heat tolerance along the coasts and in the Urban zone. These results are contrary to my prediction that ants in the coastal regions would increase their heat tolerance relative to inland sites. Greater CT_{max} in the Niagara Frontier and Southern Tier aligned with an average temperature increase in WNY throughout the summer of 2020 as is typical of summers in the region (Cedar Lake Ventures 2022, Vermette 2020). Decreasing ant CT_{max} in the Urban, Erie Coastal, and Ontario Coastal zones was contrary to seasonal warming climatic trends. In the case of *A. picea* CT_{max} increased in the Niagara Frontier, Southern Tier, and Erie Coastal zones, while decreasing in the Urban and Ontario Coastal zones. *L. americanus* did not become more heat tolerant as the season progressed.

Ants across all species increased their physiological tolerance for cold as the summer progressed. The trend of decreased CT_{min} by date also held true for both target species, *A. picea* and *L. americanus*. It is possible decreasing CT_{min} throughout the summer may be an adaptive response which occurred irrespective of summer warming. Whereas CT_{max} varied with both

season and climate zone, CT_{\min} decreased irrespective of climate zone, indicating regional climate variation did not contribute to this response. That this shift occurred across all species implicates a more ubiquitous cause, such as the annual decrease in temperature between summer and autumn. I could find no previous research to support a causal link between lowered CT_{\min} throughout the summer and cooler autumn temperatures, however, sampling extended into the final weeks of summer and concluded one week before autumn began. Average temperatures in WNY peak in July before tapering off towards the end of August and can be relatively cool in September (National Oceanic and Atmospheric Administration 2022). Thus, sampling preceded colder seasonal conditions. Most temperate ant species hibernate for survival during the colder months (Lőrinczi 2016). For example, *Aphaenogaster rudis*, a close relative of *A. picea*, begins hibernation in the early autumn (Talbot 1951). A gradual decrease in CT_{\min} throughout the summer indicates the physiological processes to prepare ants for cold temperatures and hibernation may begin earlier in the season and occur gradually.

A slight increase in femur length across all species throughout the season supported the hypothesis that femur length is plastic, however to a lesser degree than physiological thermal tolerance. Contrary to my prediction that urban ants would exhibit greater femur lengths, femurs were slightly shorter for urban ants, and climate zone had minimal relevance in predicting femur length. The trend of increasing femur length was more pronounced in *A. picea*, an already distinctly long-legged species. *L. americanus* did not increase in femur length throughout the season. Brood production is constant throughout the summer season (Heinze and Tsuji 1995) and developmental conditions as influenced by seasonal climate are also variable, thus potentially leading to plasticity between brood. However, the pronounced difference in plasticity between morphological and physiological thermal traits implies a different mechanism causes plasticity

for each type of thermal trait. A likely explanation for why morphological femur length was less plastic than physiological thermal tolerance traits is that physiological plasticity occurred in adults, whereas morphological plasticity was restricted to occur in developmental life stages. Whereas the plasticity of physiological thermal tolerance traits in adults can be explained through the upregulation of HSPs or other such metabolic substances, no such mechanism exists to regulate femur length in adults. Alternatively, femur lengthening throughout the season may be an evolutionary response to climatic variability late in the season and could lag the corresponding decrease in CT_{\min} because of the time needed to reach adulthood.

Plasticity of CT_{\max} in ant species is species-specific (Bujan et al. 2020). In some species, such as *Prenolepis imparis*, the presence or absence of plasticity in physiological thermal tolerance traits may vary intraspecifically (Tonione et al. 2020). Regional differences in plasticity of thermal tolerance traits within a species, such as with CT_{\max} in *A. picea*, suggest not all factors contributing to seasonal plasticity are accounted for or understood. Variation in seasonal plasticity between WNY climate zones suggests seasonal plasticity may be partially dependent on factors such as those that contribute to climatic variation between WNY climate zones, such as land use, elevation, or distance from a large body of water. Investigating the source of this variation between geographically close populations of a species may yield evidence of the underlying mechanisms behind thermal acclimation.

An underlying mechanism may connect tolerance for adverse heat and cold in some ant species sampled. For instance, a tradeoff exists in *A. picea* wherein increased cold tolerance limits overall range of thermal tolerance (Nguyen et al. 2019). This tradeoff implies physiological mechanisms controlling ant cold and heat tolerance could in some way be connected and could explain why CT_{\max} decreased across all species in the Erie Coastal, Ontario

Coastal, and Urban WNY climate zones. Whereas the Ontario Coastal and Erie Coastal WNY climate zones tend to have lower minimum temperatures in the spring, the Urban WNY climate zone tends to be warmer than its surroundings at all times of the year (Vermette 2020). However, ants in the Urban WNY climate zone do not benefit from the moderating effects of forest canopy cover on microclimate, unlike those in the more densely wooded Niagara Frontier and Southern Tier WNY climate zones (Martini et al. 2020, Vermette 2020). Because ants in the Erie Coastal, Ontario Coastal, and Urban WNY climate zones were exposed to more severe cold in the spring, their ability to acclimate to heat may have been compromised. Further investigation to pin down the mechanism controlling seasonal plasticity in ants should thus not neglect CT_{min} when elucidating the underlying physiology. Incorporating CT_{min} into these investigations could offer insight into whether or not heat and cold tolerance interact or are otherwise dependent on one another. Alternatively, because climate models are based on air temperature and tend to underpredict the temperature of soil before green out (Vermette and Graves 2015), ants in the Erie Coastal, Ontario Coastal, and Urban WNY climate zones may have been responding to elevated soil temperature, for which no data was recorded. Elevated soil temperature could be more relevant to open canopy sampling sites, for which no target species could be selected, and the success of sampling was relatively less than closed canopy sampling sites. Further investigation should thus not neglect soil temperature as a variable and should compare closed canopy and open canopy sampling sites.

Evidence of seasonal plasticity was absent in *L. americanus* for both CT_{max} and femur length, in contrast to *A. picea*. It is possible that *L. americanus* is physiologically and morphologically incapable of plasticity in these traits and mitigates climatic variation in some other way, such as behaviorally. These results emphasize that seasonal plasticity is a species-

specific phenomenon in ants, and so the biology and ecology of each species should be considered individually when investigating seasonal plasticity.

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Figures

Figure 1a: Plot of CT_{min} as a function of Julian date for all species.

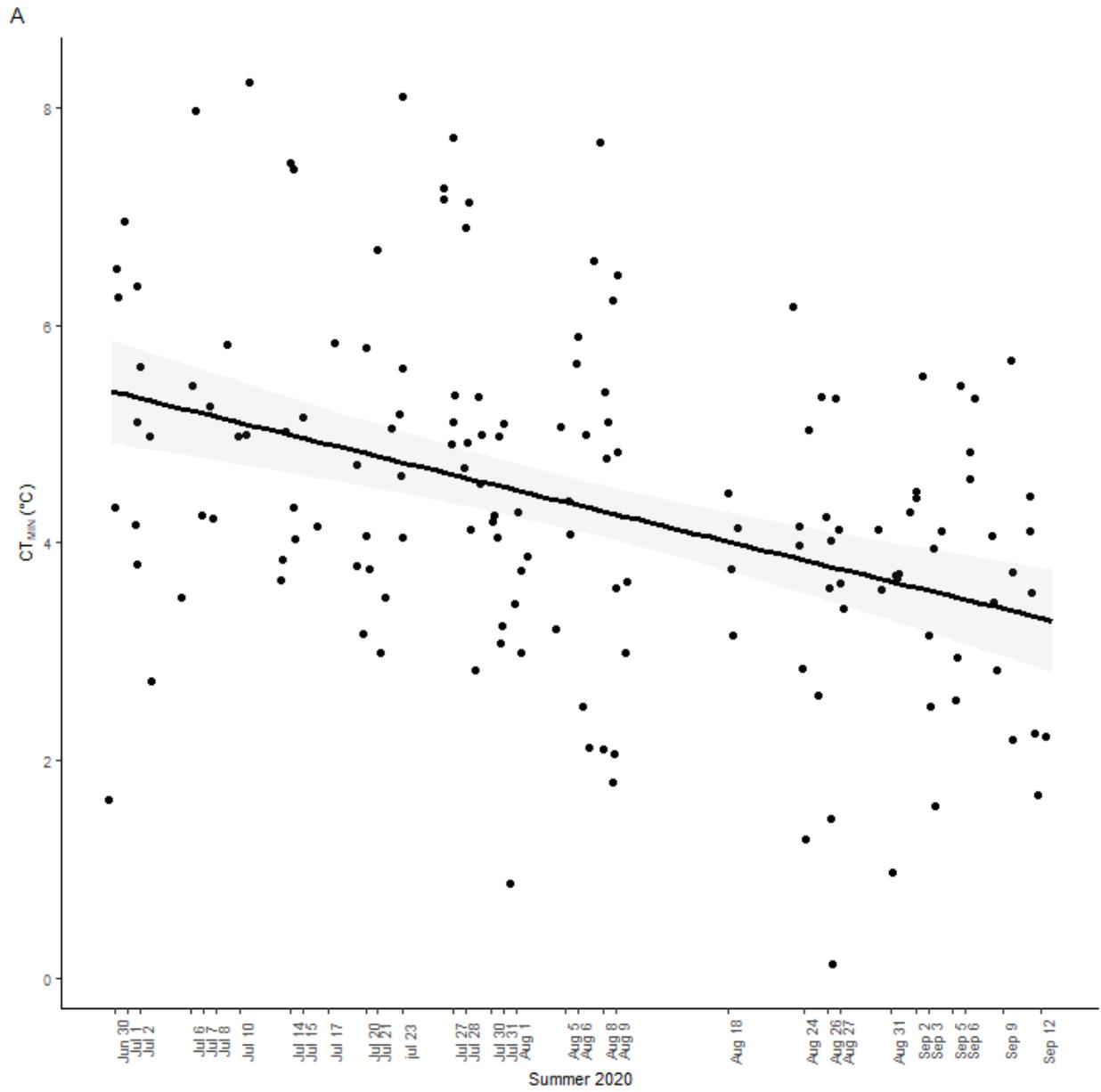


Figure 1b: Box and whisker plot for CT_{min} of all species.

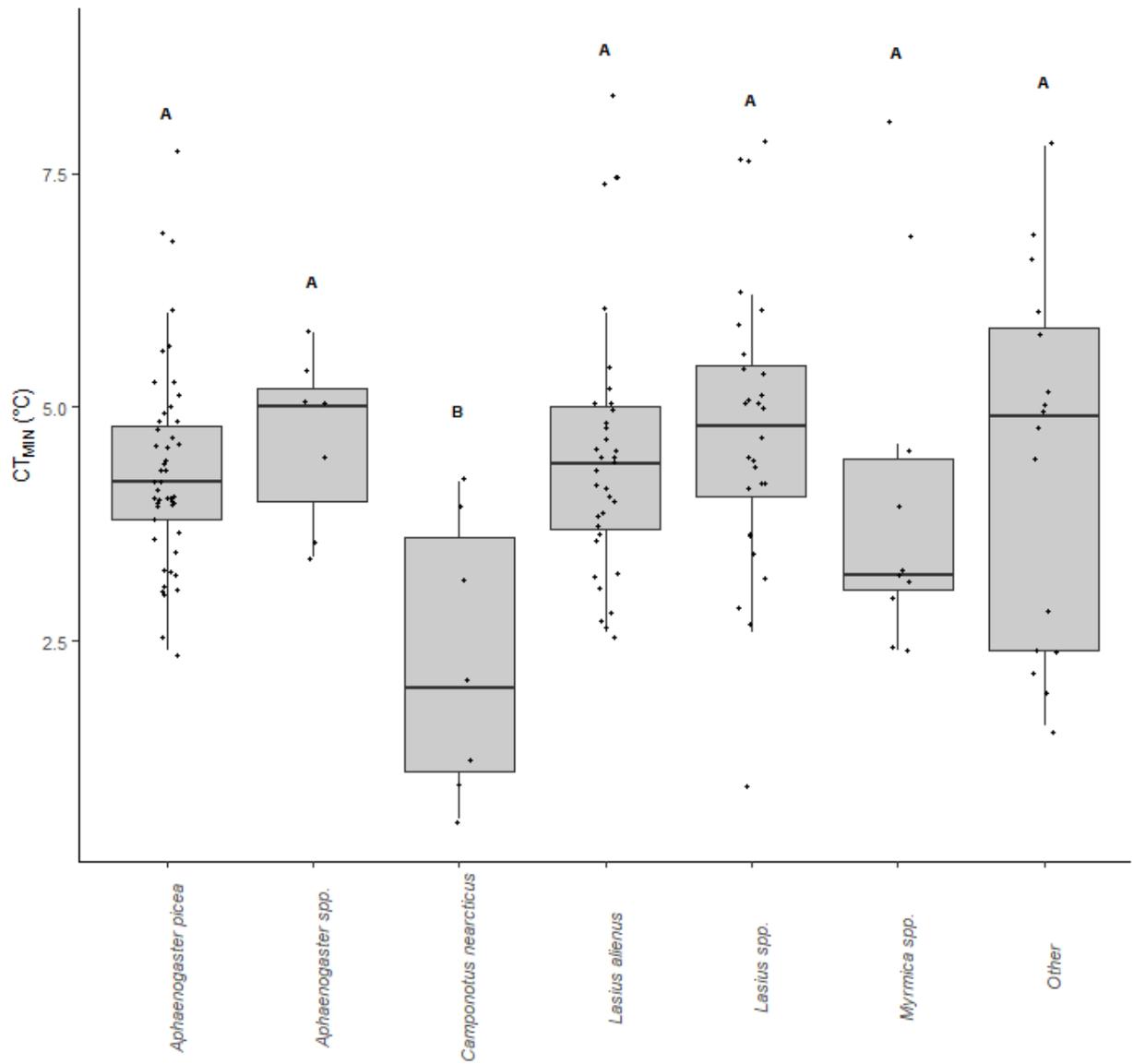


Figure 2a: Continuous interaction plot for CT_{MAX} as a function of Julian date x WNY Climate zone across all species.

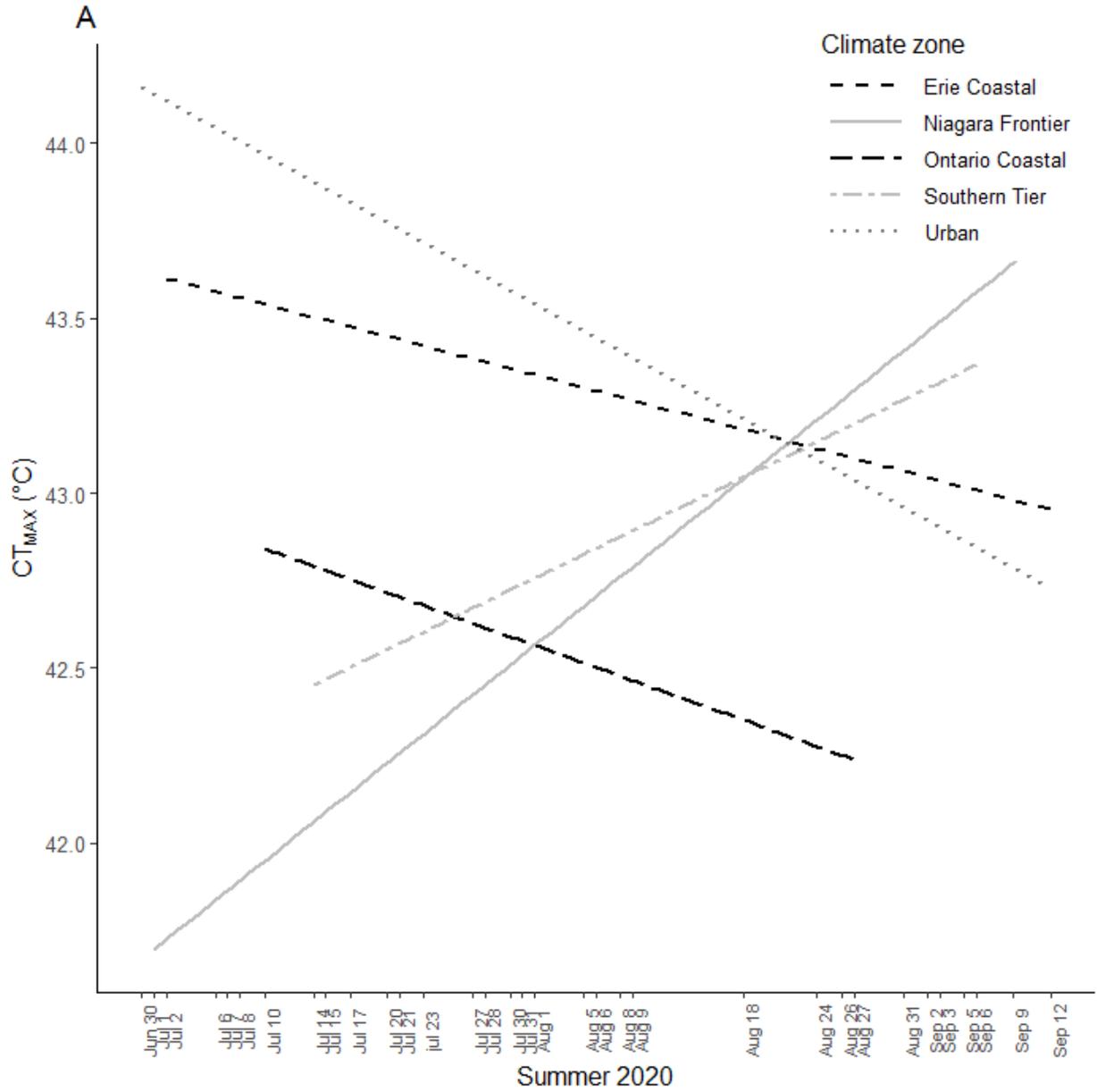


Figure 2b: Box and whisker plot for CT_{max} of all species.

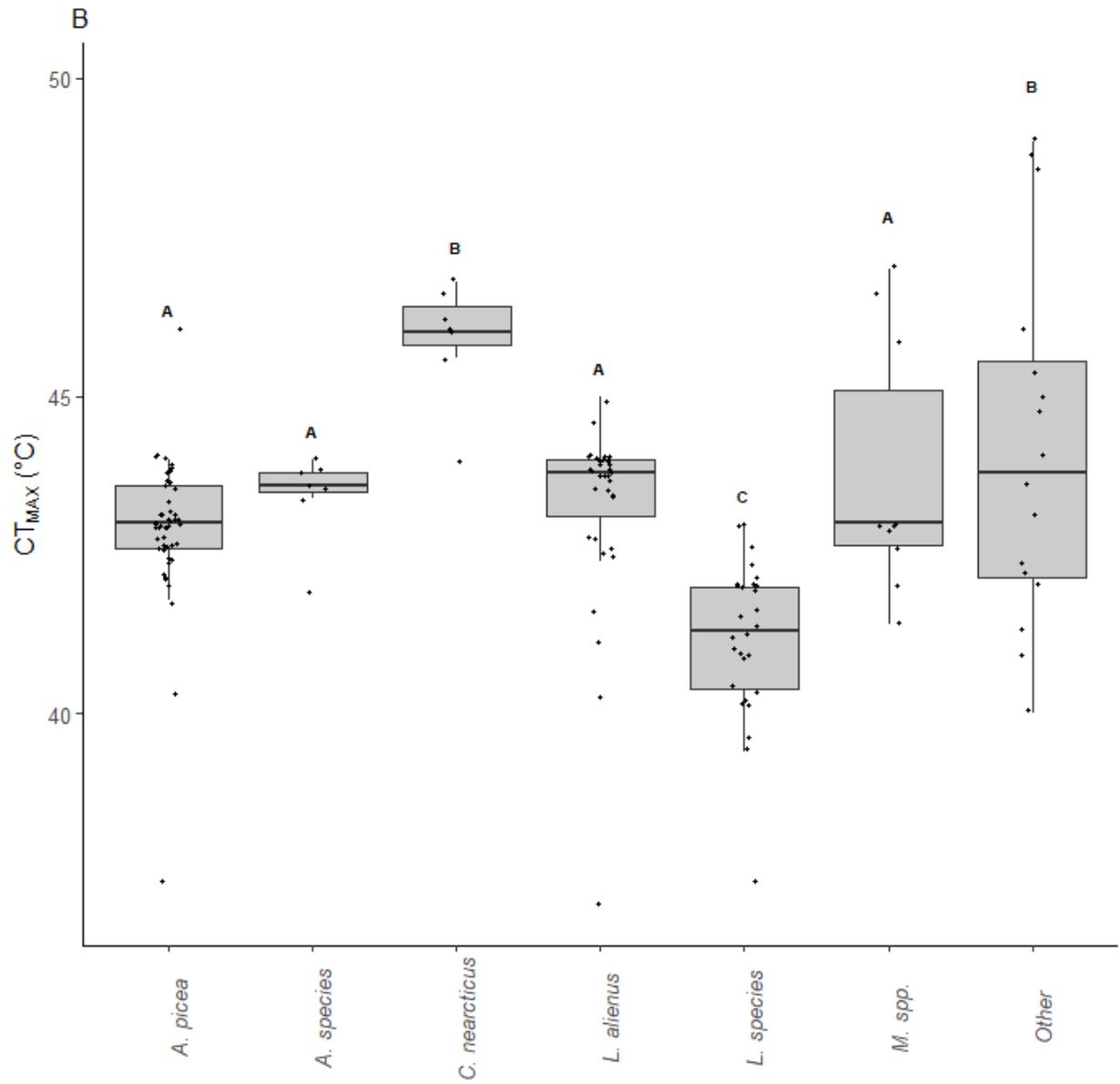


Figure 3a: Plot of femur length as a function of Julian date for all species.

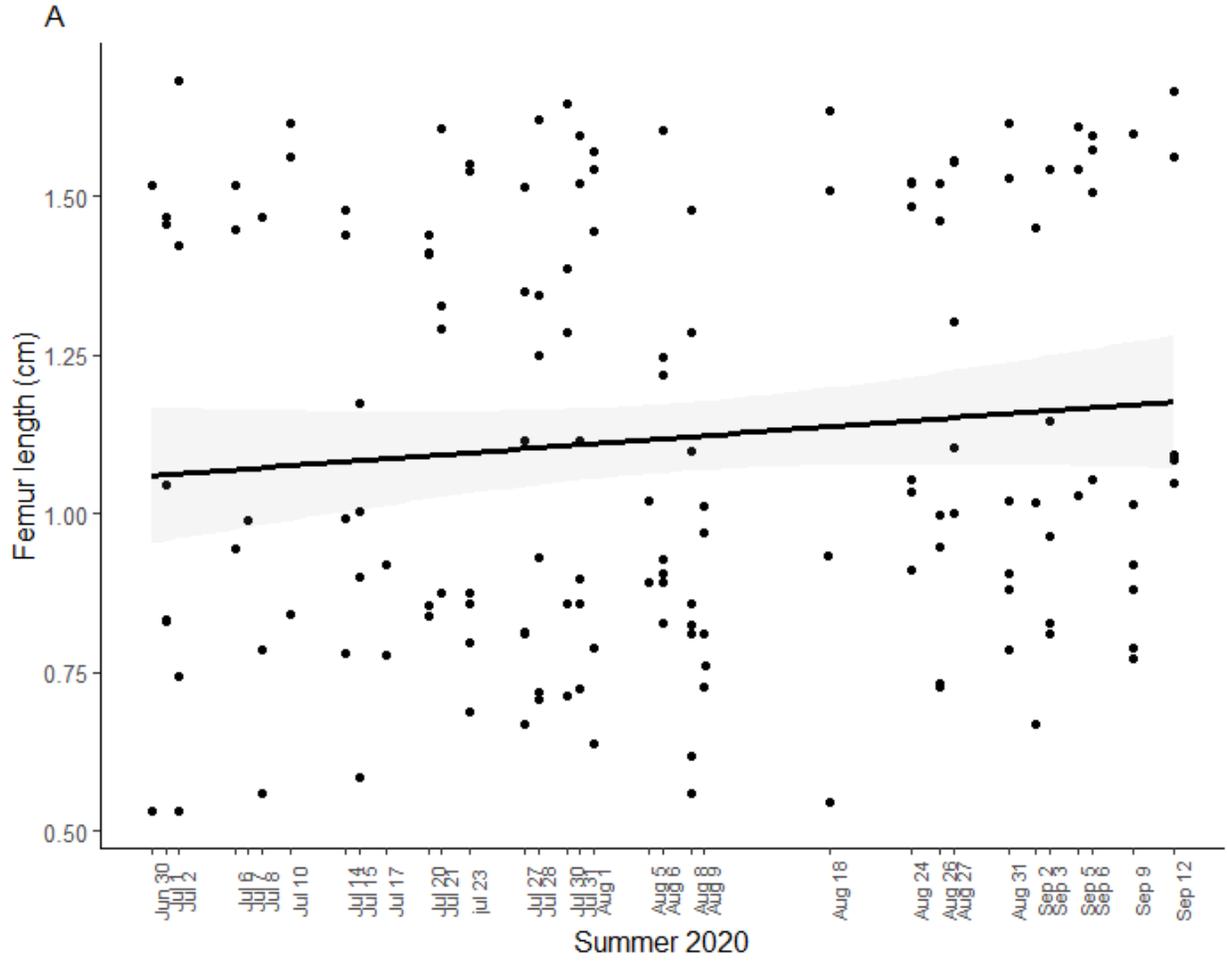


Figure 3b: Box and whisker plot for femur length of all species.

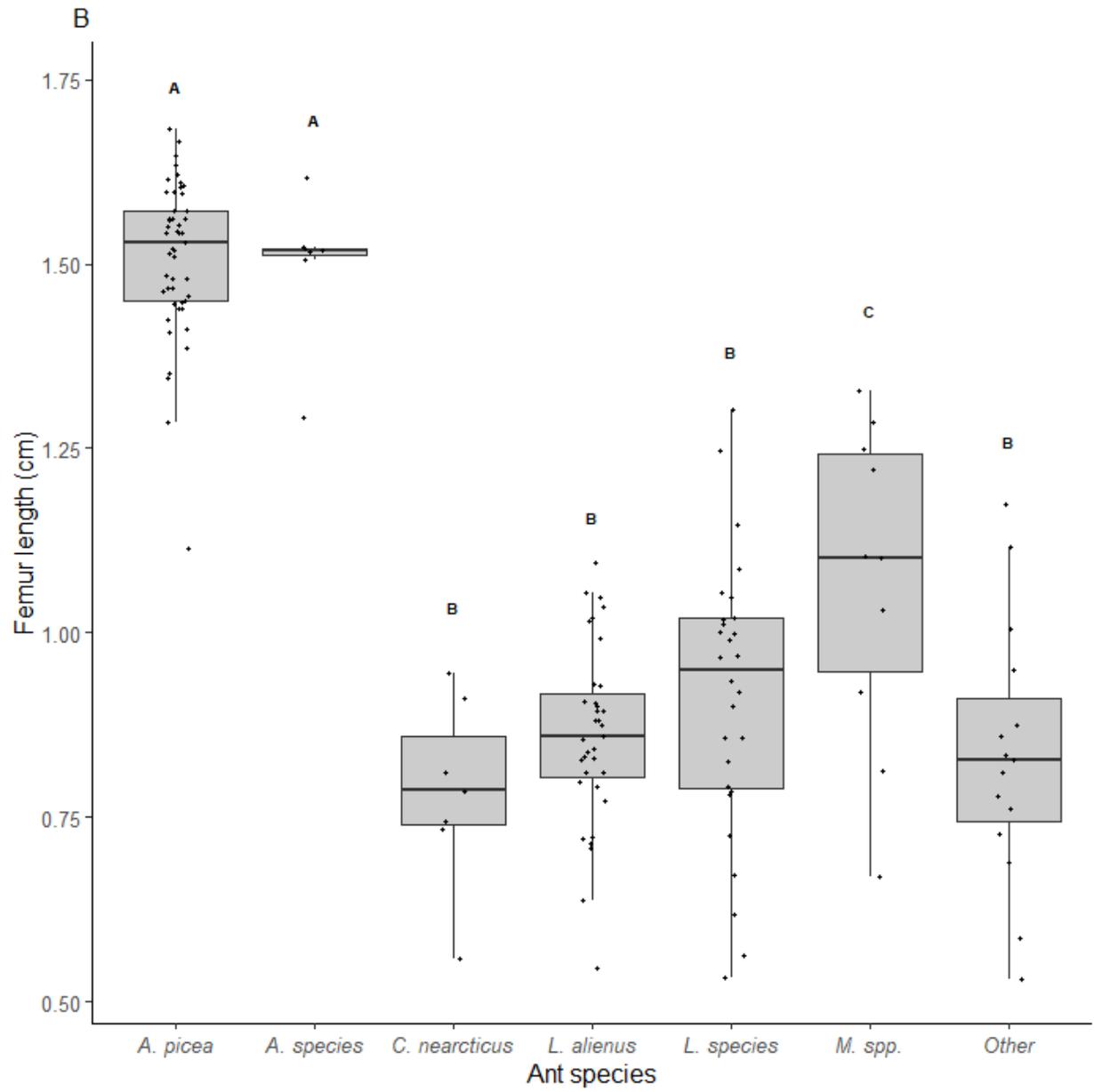


Figure 4: Fitted linear regression with standard error shaded showing the decrease of CT_{min} in *Aphaenogaster picea* as a function of Julian day.

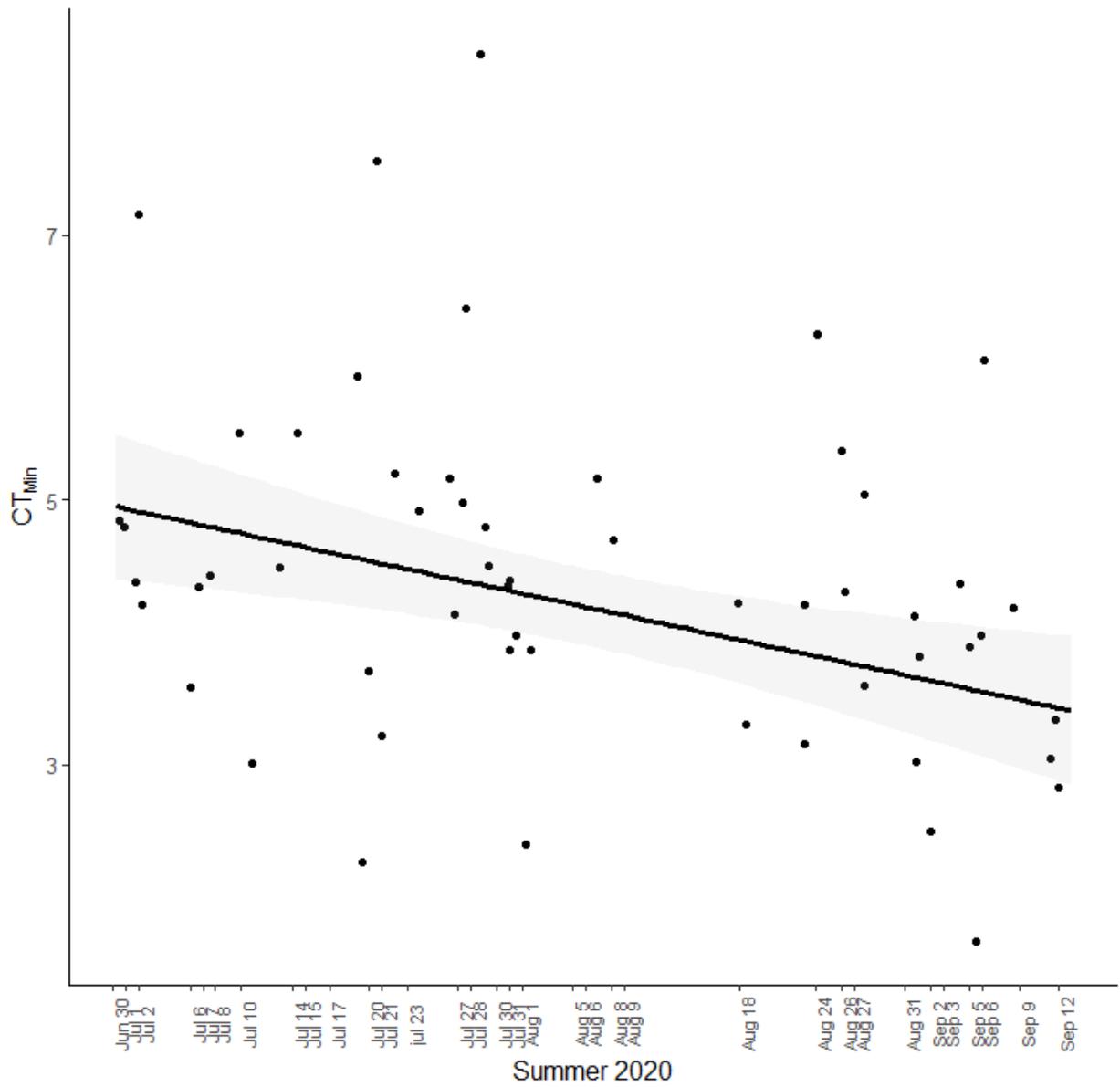


Figure 5: Continuous interaction plot for CT_{max} as a function of Julian date x WNY Climate zone for *Aphaenogaster picea*.

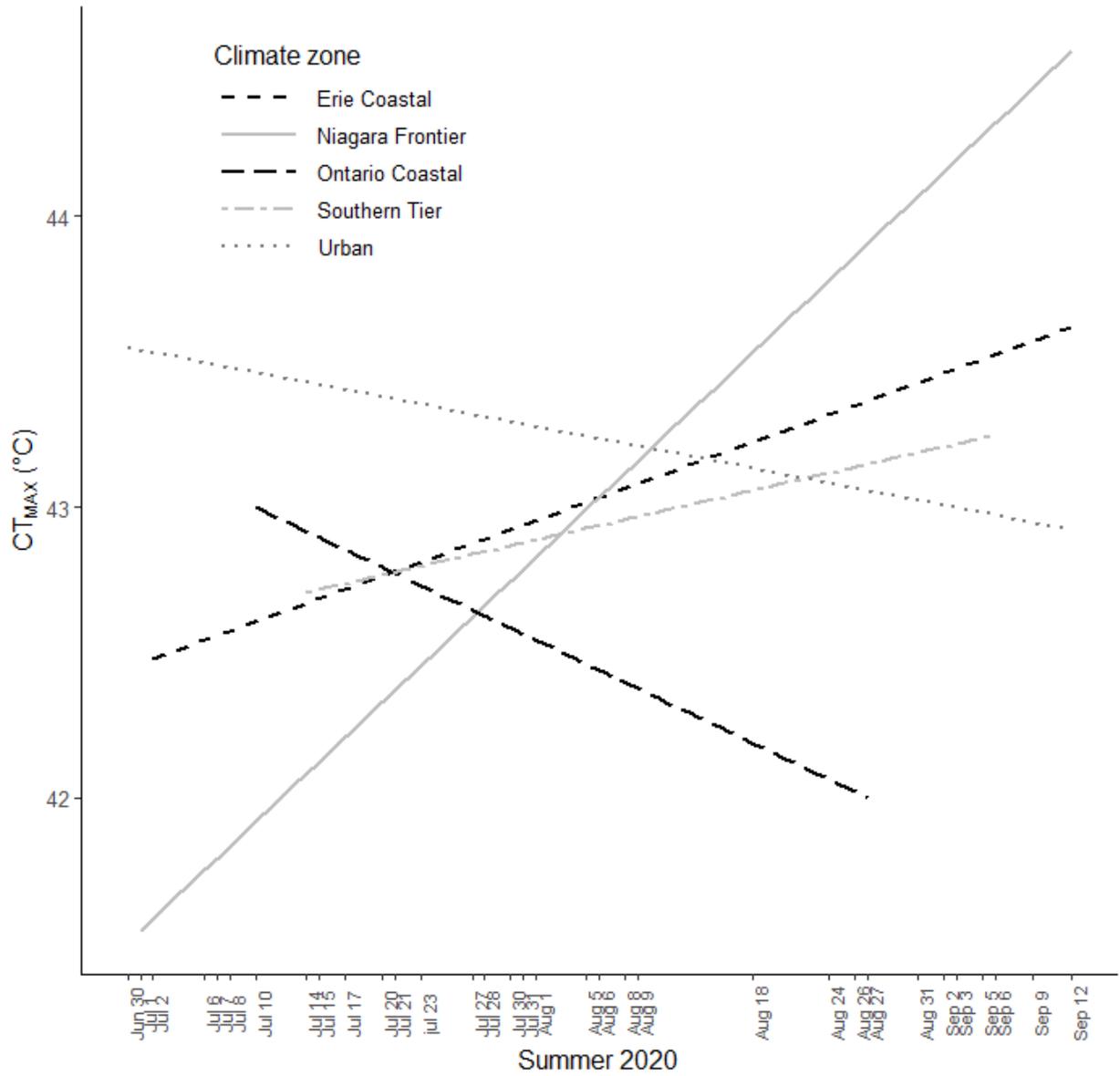


Figure 6: Fitted linear regression with standard error shaded showing the increase of femur length in *Aphaenogaster picea* as a function of Julian day.

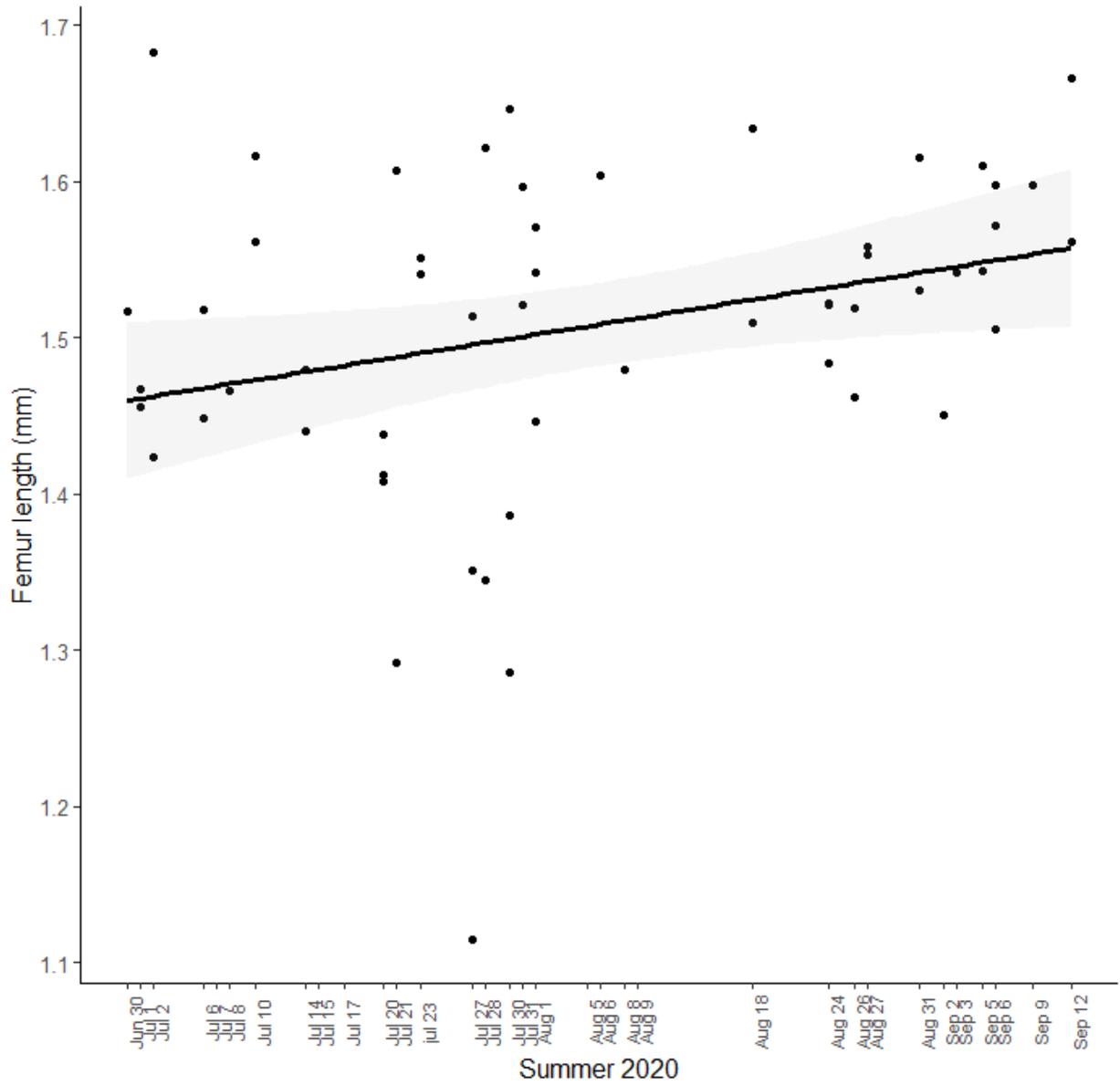
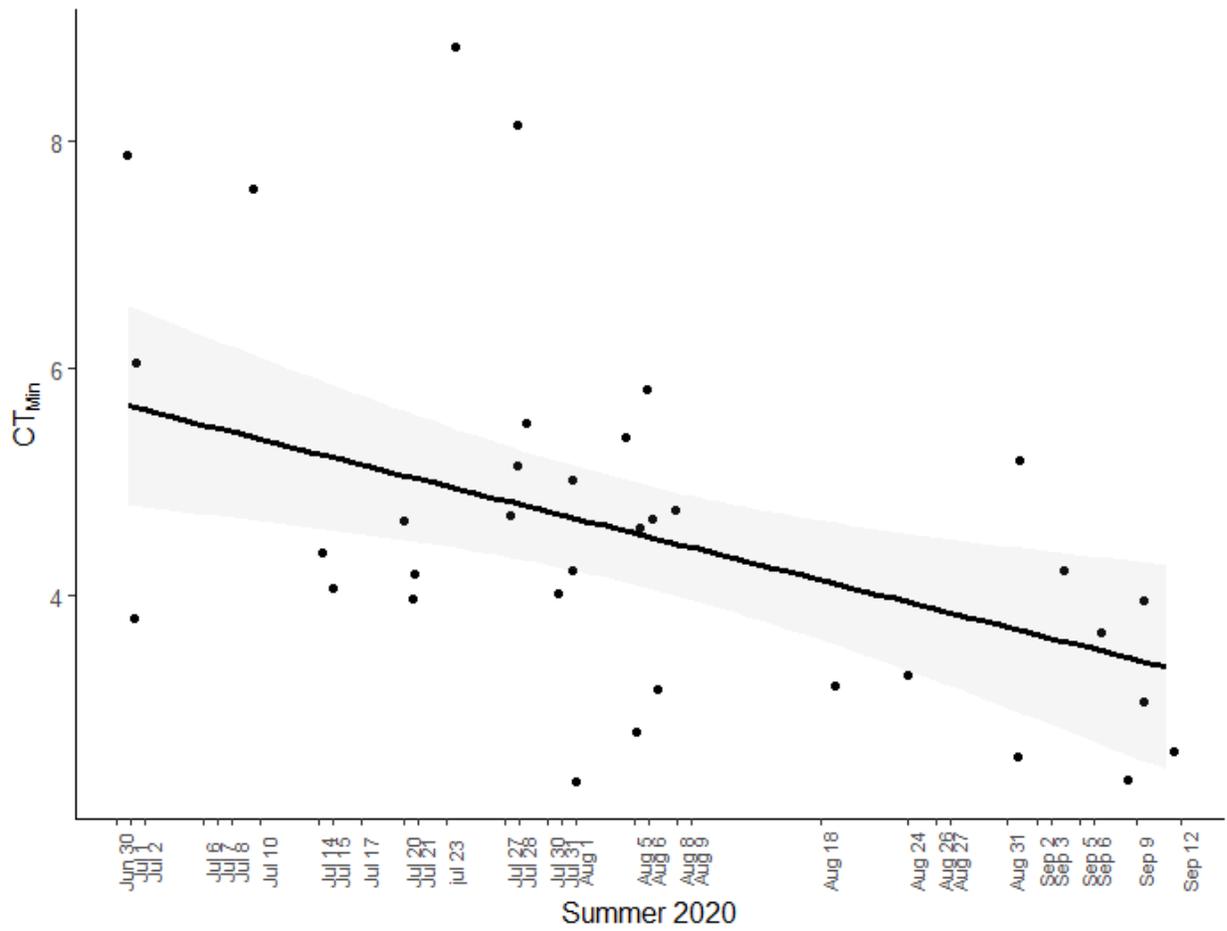


Figure 7: Fitted linear regression with standard error shaded showing the decrease if CT_{min} in *Lasius Americanus* as a function of Julian day.



Appendix

Figure 8: Map of first sampling series with overlay of WNY climate zone map from Vermette (2020), created with Google Earth.

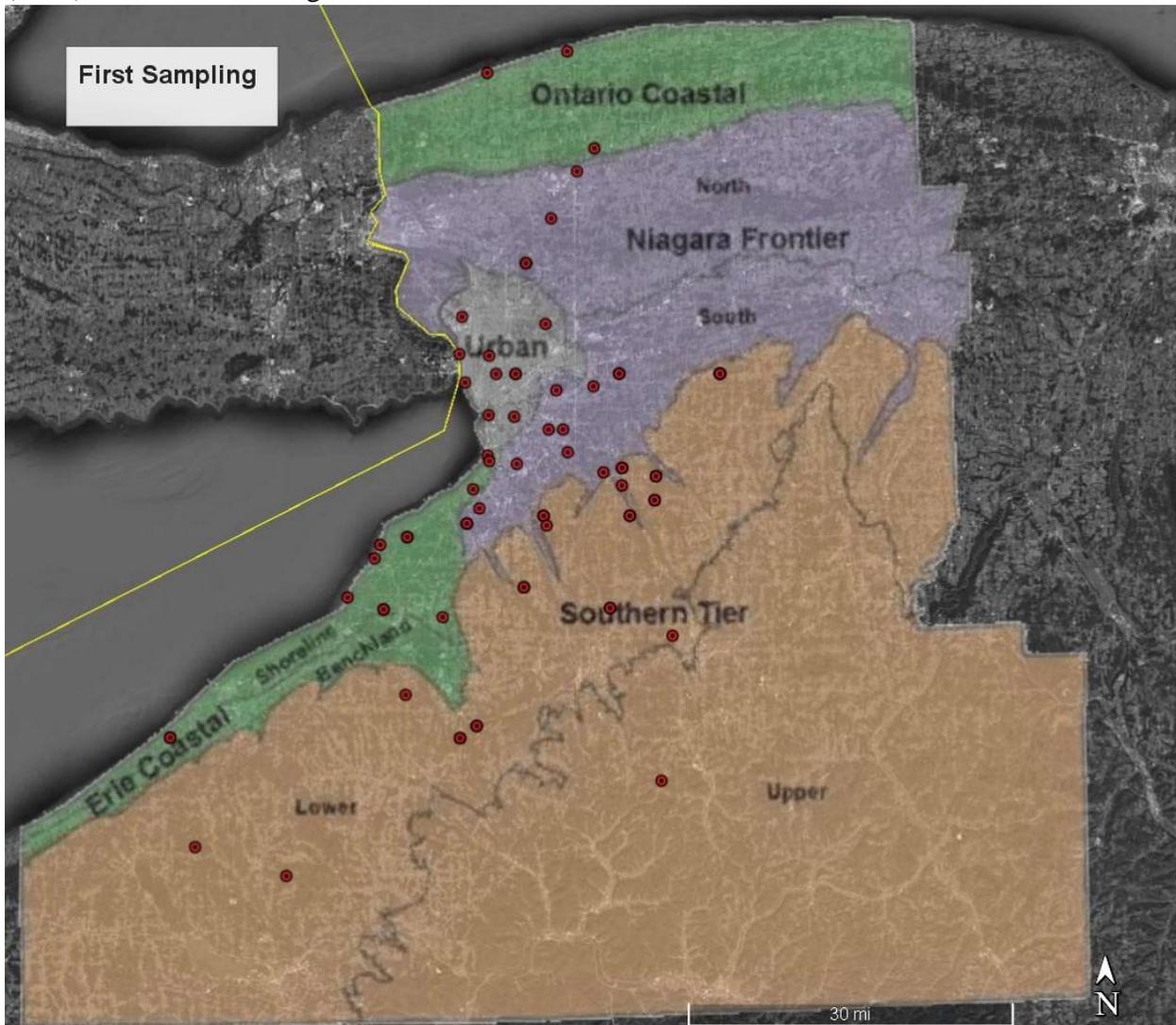


Figure 9: Map of second sampling series with overlay of WNY climate zone map from Vermette (2020), created with Google Earth.

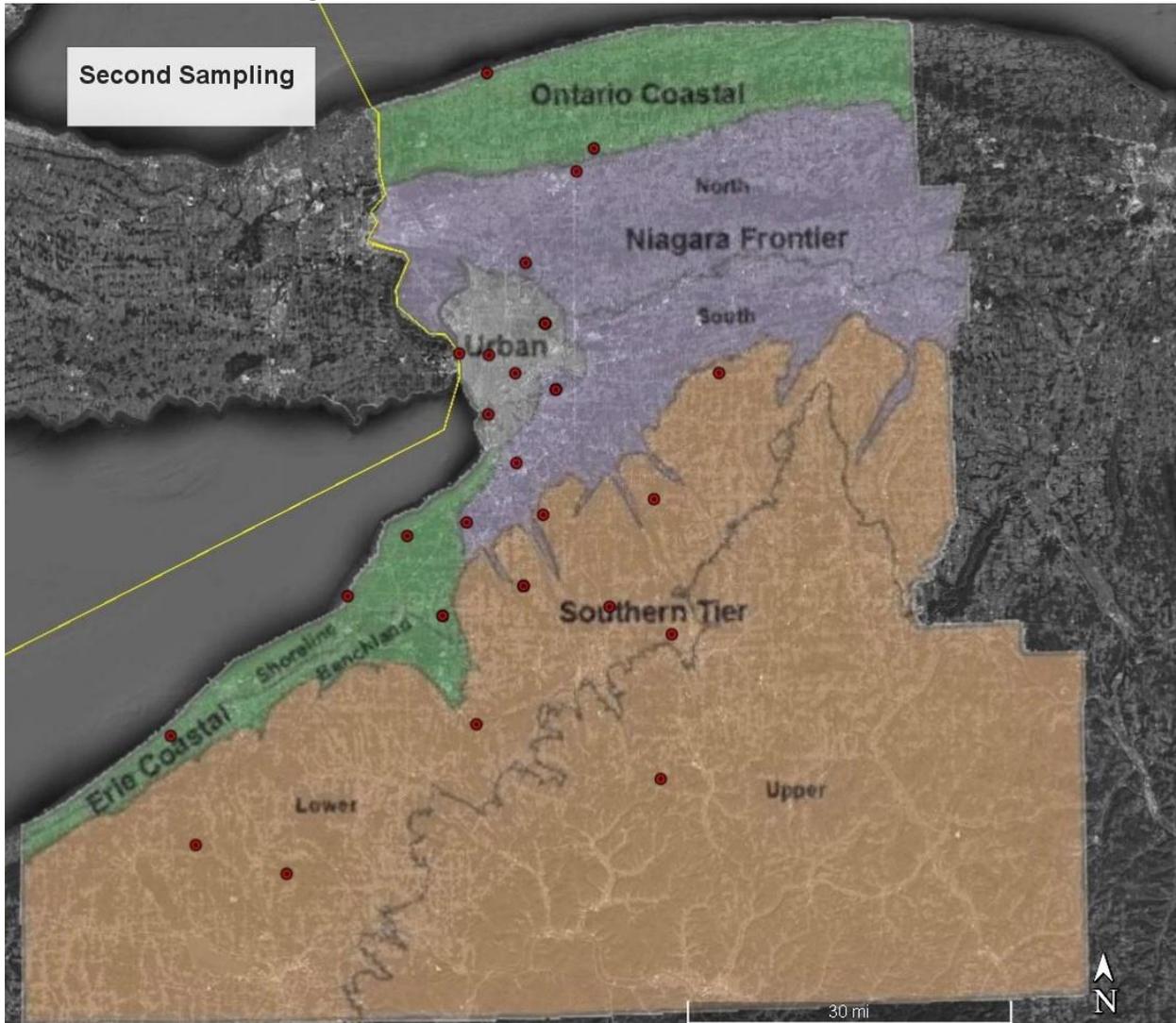


Figure 10: Stereomicroscope image of *A. picea* used to obtain morphometric data.



Figure 11: Stereomicroscope image of *L. americanus* used to obtain morphometric data.

