Climate-driven range shift prompts species, not gene, replacement

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<td>Abstract:</td>
<td>Climate change prompts warm-tolerant species upward and poleward to either displace or replace cold-tolerant species. Warm-tolerant species may replace cold-tolerant individuals with upward migration, or cold-tolerant genes if the species hybridize. We examined morphological traits and genetic introgression between low elevation warm- and high elevation cold-tolerant ant species that form an upward-shifting ecotone in the southern Appalachian Mountains (U.S.). The ant ecotone shifted upward ca. 200 m between the decades 1970 and 2010, but distinguishing morphological traits of the high and low elevation ants were muddled where the species met, suggesting hybridization. However, we found no evidence of genetic hybridization, and the distinguishing traits were more strongly linked with physiological cold tolerance and environment than species identity. These results indicate that the cold tolerant ant species, associated with high-elevation and high-latitude, was replaced by the warm-tolerant, low elevation ant species. Interestingly, once at higher elevations, the warm-tolerant species exhibited phenotypic plasticity so that it morphologically resembled the displaced cold-tolerant species.</td>
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Title: Climate-driven range shift prompts species, not gene, replacement

Running head: Climate-driven range shift

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Climate change prompts warm-tolerant species upward and poleward to either displace or replace cold-tolerant species. Warm-tolerant species may replace cold-tolerant individuals with upward migration, or cold-tolerant genes if the species hybridize. We examined morphological traits and genetic introgression between low elevation warm- and high elevation cold-tolerant ant species that form an upward-shifting ecotone in the southern Appalachian Mountains (U.S.). The ant ecotone shifted upward ca. 200 m between the decades 1970 and 2010, but distinguishing morphological traits of the high and low elevation ants were muddled where the species met, suggesting hybridization. However, we found no evidence of genetic hybridization, and the distinguishing traits were more strongly linked with physiological cold tolerance and environment than species identity. These results indicate that the cold tolerant ant species, associated with high-elevation and high-latitude, was replaced by the warm-tolerant, low elevation ant species. Interestingly, once at higher elevations, the warm-tolerant species exhibited phenotypic plasticity so that it morphologically resembled the displaced cold-tolerant species.

**Keywords:** Aphaenogaster, rudis complex, hybridization, traits, phenotypic plasticity, phylogenetics
Introduction

Species generally move upward and poleward with climate warming (Brommer 2004; Parmesan et al. 1999; Pelini et al. 2009; Zuckerberg et al. 2009). If all species move in the same direction at the same rates, their ranges and interactions would simply shift in space without increasing overlap and interaction pressures. However, species move at different rates in response to climate change (Ibanez et al. 2008; Kelly and Goulden 2008; Miller-Rushing et al. 2008; Pelini et al. 2009; Perry et al. 2005; Schweiger et al. 2008; Zhu et al. 2013) so that competitive interactions may increase (Urban et al. 2012; Zarnetske et al. 2012). Competitive interactions are most intense between closely related species that share similar resource requirements (Darwin 1859; Gause 1934; Hardin 1960) so that less competitive (cold-tolerant) species may be replaced by more competitive (cold-intolerant) species with warming (Connell 1975; Menge and Sutherland 1987; Urban et al. 2012). Alternatively, closely related species may interbreed so that genes rather than species are replaced, and hybrid zones move upward and poleward (Buggs 2007; Harr and Price 2014; Wolf et al. 2001).

Boreal forest communities similar to those in the northeastern United States and Canada blanketed the southern Appalachian Mountains during the most recent glacial maximum ~18,000 years ago (Delcourt and Delcourt 1987; Delcourt and Delcourt 2000). As the Pleistocene ended, species adapted to cold climates migrated northward, and in montane environments, upward. Generally, species that can better tolerate stressful conditions (e.g., cold) are poor competitors (Chase and Leibold 2003; Tilman and Pascala 1993), and better competitors may replace cold tolerant species with climate change (Urban et al. 2012). High elevation species not always are replaced, however, and in some cases form hybrid zones with low elevation congeners. For example, ecotones between closely related high- and low-elevation salamander species persist in
the Southern Appalachian Mountains (U.S.), and, though their interactions along elevation
gradients generally are competitive, they hybridize where distributions overlap (Bruce 2007;

Ant congeners commonly hybridize (Cahan and Keller 2003; Julian et al. 2002;
Shoemaker et al. 1996), and ant complexes can be very cryptic (Seifert 2009), with plastic
morphology potentially obscuring species differentiation across environmental gradients (e.g.,
darker colors with increased elevation) (MontBlanc et al. 2007). For example, in eastern North
America (N.A.), approximately 12 Aphaenogaster species are the most abundant arthropod in
mesic deciduous forests (King et al. 2013), but eastern N.A. Aphaenogaster species are hard to
differentiate based on morphology (Lubertazzi 2012; Ness et al. 2009; Umphrey 1996), and their
taxonomy remains unresolved (Creighton 1950; Crozier 1977; Lubertazzi 2012; Seifert 2009;
Umphrey 1996), particularly 3-6 species (including A. rudis and A. picea; hereafter ‘rudis
complex’). Still, previous genetic, geographic and ecological data suggest discrete species with
distinct, albeit overlapping, N.A. distributions (Crozier 1977; Umphrey 1996; Warren II et al.
2011b).

Ant species, including Aphaenogaster, typically partition habitat (spatially and
temporally) by temperature (Dunn et al. 2007; Parr and Gibb 2009; Sanders et al. 2007). In the
southern Appalachian Mountain region (U.S.), two Aphaenogaster species appear to segregate
latitude and altitude by minimum temperatures (Warren II et al. 2011a; Warren II and Bradford
2013; Warren II and Chick 2013; Warren II et al. 2011b). The northerly, high-elevation A. picea
forages at temperatures ca. 6°C below that of the southerly, low-elevation A. rudis (Warren II et
al. 2011a), and experimental studies indicate that A. picea's physiological cold tolerance is ca.
2°C lower than A. rudis (Warren II and Chick 2013). The ecotone between A. rudis and A. picea
shifted upward and northward with rising minimum temperatures in the southern Appalachian Mountain region 1974-2012 (Warren II and Chick 2013). In measuring the A. rudis/A. picea ecotone across elevation gradients, Warren II and Chick (2013) noted that the species were difficult to differentiate at mid-elevations based on morphological characters, and suggested that the two closely related species might hybridize. However, in investigating the same elevation gradients, Crozier (1977) found little chromosomal evidence of hybridization.

We used genetic and morphometric analysis of these ant samples (Warren II and Chick 2013) and additional A. rudis and A. picea samples (King et al. 2013) collected ca. 75 km north and 75 km south of the northern Georgia ecotone to investigate whether the shift in species was caused by replacement or hybridization. If replacement occurred, we would expect no genetic evidence of hybridization and a discrete 'step' in species traits with transition between cold-intolerant, low-elevation A. rudis and cold-tolerant, high-elevation A. picea. If hybridization occurred, we would expect evidence of genetic introgression and a continuous shift in traits across the species boundary. We also investigated links between morphological traits that potentially differentiate the Aphaenogaster species and their thermal tolerances.

Methods

Specimen collections

In 2011, A. rudis was collected at Whitehall Forest (WHF) in Athens, GA, and A. picea was collected at Coweeta Hydrologic Laboratory (CWT) in Otto, NC (for methodology and GPS locations see King et al. 2013). In 2012, A. rudis (low elevation) and A. picea (high elevation) ants were collected in northern Georgia (NGA) in Chattahoochee National Forest and Black Rock Mountain State Park (for methodology and GPS locations see Warren II and Chick 2013).
The ant samples were dried and stored frozen at 4°C until 2013 when they were pinned and mounted for morphometric analysis. A total of 162 specimens from 2011 and 54 specimens from 2012 were used. Voucher specimens are stored at the Georgia Museum of Natural History (GMNH) and SUNY Buffalo State's insect collection. Location data and morphometric measurements also were taken from voucher specimens collected by Crozier (1977) [CRO] and stored at the GMNH.

**Morphometrics**

Morphometric measurements were taken using a Leica M125 stereomicroscope with DFC295 digital camera and Leica Application Suite V4. Five traits were measured: head width (HW, maximum width of head including outer edges of eyes), head length (HL, maximum length of head excluding mandibles), eye width (EW, maximum eye width), eye length (EL, maximum eye length) and femur length (FL, maximum femur length, posterior view). Given that *A. rudis* ant legs typically are lightly colored and reddish and *A. picea* legs darker black, and that *A. rudis* antenna segments are of uniform color whereas the last four segments of *A. picea* antenna are lighter (Ellison et al. 2012), color and antenna indices were generated. The leg color index (LEG) was coded as 1 = light, 2 = medium, 3 = dark; the antenna index (ATN) was coded as 1 = segments uniform, 2 = last four segments somewhat lighter than rest, 3 = last four segments clearly lighter than rest. Two technicians not involved in Warren II and Chick (2013) independently coded index specimens.

**Genetics**
Aphaenogaster specimens were sent to the A.J. Cook Arthropod Research Collection, Michigan State University, East Lansing, Michigan, using an overnight mail carrier from northern Georgia (A. rudis) and western North Carolina (A. picea). Six of these specimens from NGA and CWT were included in a phylogenetic analysis using DNA data from five genes including the mitochondrial gene cytochrome oxidase subunit 1 (CO1), and nuclear genes: carbamoylphosphate synthase (CAD), elongation factor 1-alpha (EF1αF2), Long-wavelength Rhodopsin (LWR) and Wingless (WG). A total of 52 specimens were used in this study (Table 1). All methods for DNA extraction, amplification and PCR (polymerase chain reaction) were followed using methods from De Marco and Cognato (in press). Primers used in PCR came from Brady et al. (2006) and Ward et al. (2010). After PCR, unincorporated deoxyribonucleotide triphosphates (dNTPs) and oligonucleotides were removed from PCR reactions with Exo-SAP (http://www.usbweb.com/category.asp?cat=pcr&id=78200) and directly sequenced on an ABI 3700 automated sequencer using a BigDye (Applied Biosystems, Inc., Foster City, CA) fluorescent chemistry reaction. Both sense and anti-sense strands were sequenced for all individuals. These samples were analyzed as part of a larger project to create a multiple gene phylogeny for Aphaenogaster in North America (De Marco and Cognato in press). This phylogeny was inferred with Bayesian analysis with Mr. Bayes via the CIPRES Gateway (Huelsenbeck and Ronquist 2001; Miller et al. 2010). Data were partitioned by gene and codon position (Castoe et al. 2004), with models of evolution applied independently to each partition (Nylander et al. 2004), with a best-fit GTR + I + G model, 20 million generations and a burn-in of 5,000,000 generations. Samples of A. carolinensis and A. miamiana were included in the phylogeny due to an overlap of geographic locations and some morphological characters. Aphaenogaster carolinensis
and *A. miamiana* can be found in North Carolina, along with *A. rudis* and *A. picea*.

*Aphaenogaster miamiana* was previously known only from Florida (De Marco and Cognato in press), but can be distinguished from the others based on heavier sculpturing.

**Climate and thermal tolerance**

Mean maximum ($T_{\text{max}}$) and minimum ($T_{\text{min}}$) temperatures, and precipitation (Prec), for the period June 2011 to June 2012 were calculated for each ant collection site (based on GPS coordinates) using PRISM Climate Data ([http://www.prism.oregonstate.edu](http://www.prism.oregonstate.edu)). For ant thermal tolerance, we used the minimum ($CT_{\text{min}}$) and maximum ($CT_{\text{max}}$) physiological temperature tolerances for *A. rudis* and *A. picea* ants collected in NGA in 2012 as part of Warren II and Chick (2013). We transferred individuals to 16mm glass test tubes that were plugged with cotton to reduce thermal refuges and placed the test tubes in an Ac-150-A40 refrigerated water bath (NesLab, ThermoScientific). One vial contained only a copper-constantan Type-T thermocouple (Model HH200A, Omega, Connecticut, USA) to monitor temperature fluctuation inside the test tubes and ensure an accurate temperature reading at which individuals reached their thermal limits. We measured thermal tolerance for 10 individuals (5 for $CT_{\text{min}}$, 5 for $CT_{\text{max}}$) from each colony at each site. We estimated thermal tolerance by determining the loss of righting response as the index for thermal tolerance and calculated the mean tolerance temperature for each species at each site. See Warren II and Chick (2013) for full methodology.

**Data analysis**

We examined variation among ant traits, physiological tolerance, and climate variables using principal component analysis (PCA) using the “prcomp” method and “scale” option.
(standardizes all variables to unit length) in the “R” statistical package (R Development Core Team 2015). Because several of the traits were highly correlated, we combined the LEG and ATN indices (IND = LEG + ATN), and we used a common ant trait index for the head and eye traits: relative eye index (REL = EL/HW). We retained FL for further analyses because it was not correlated with the other traits.

We used linear regressions to examine the effect of elevation on IND, REL and FL in ants collected along altitude gradients in north Georgia. Given that a shift in species occurred between low-elevation *A. rudis* and high-elevation *A. picea* at ca. 750 m elevation (Warren II and Chick 2013), we included second-order terms to capture any nonlinear shifts. We also used linear regressions to examine whether the morphological and physiological ant traits were linked, as suggested by the PCA results, by testing the effect of CT\textsubscript{min} and CT\textsubscript{max} on IND, REL and FL in specimens from NGA, CWT, WHF and CRO using AIC criteria (Δ AIC < 2).

Because several traits vary with elevation, species identification at middle altitudes is less certain than at the southerly, low-elevation WHF site (*A. rudis*) and the northerly, high-elevation CWT site (*A. picea*). We used the CWT and WHF sites to examine differences in trait values between species. A generalized linear model (GLM) with a binomial error distribution was used to test whether IND, REL and FL differed between *A. rudis* and *A. picea*. Overdispersion in the GLM was < 1. We considered coefficients with a p-value < 0.05 ‘significant’ and those with a p-value < 0.10 ‘marginally significant’ (sensu Hurlbert and Lombardi 2009).

**Results**

*Phylogenetic analysis*
The phylogenetic analysis produced a mostly resolved Bayesian tree with strong support for the outgroups, including *Camponotus pennsylvanicus*, *Formica glacialis*, *Stenamma diecki*, *Novomessor cockerelli*, *Veromessor julianus* and *Solenopsis invicta* (S1). There also was strong support for the *A. fulva* and *A. picea* clades, however *A. picea* was separated into two smaller clades. The sample from North Carolina was in the smaller clade. *Aphaenogaster carolinensis* and *A. miamiana* clades each showed strong support, however they were within the less supported *A. rudis* clade. *Aphaenogaster carolinensis* and *A. miamiana* were distinguished from *A. rudis* due to a missing intron in the gene CAD.

**Principal component analysis**

Principal component analysis on the full set of ant physical and physiological traits, climate variables and elevation indicated that six principal components accounted for 96% of the variance in the 13 variables. We plotted the first two components, which accounted for 74% of the variance (Fig. 1). Prec and Elev were interchangeable, and they were negatively correlated with $T_{\text{max}}$, $C_{\text{T}_{\text{min}}}$, $C_{\text{T}_{\text{max}}}$ and FL along the PC1 axis (which accounted for 60% of the variability). EL, HW, HL and EW appeared to covary together, particularly HW and HL, which were interchangeable, and they were orthogonal to the PC1 axis, oriented more toward the PC2 axis (which accounted for 14% of the variability). ATN and LEG covaried together, were oriented toward the PC2 axis, and were negatively correlated with $T_{\text{min}}$.

**Trait variation with elevation**

IND (leg/antenna coloring index) increased significantly with elevation ($\text{coef.} = 0.006$, $SE = 0.001$, $t$-value $= 8.509$, $p$-value <0.001), meaning that the ants became darker in leg color and
last four antenna segments at higher elevations (Fig. 2a). REL (relative eye index), however, was
unaffected by elevation (coef. = -0.001, SE = 0.001, t-value = -1.039, p-value = 0.301); eye
morphometrics did not vary with elevation (Fig. 2b). FL (femur length) decreased significantly
with elevation (coef. = -1.938e-04, SE = 8.944e-05, t-value = -2.167, p-value = 0.0324), meaning
that ant legs became shorter at higher elevations (Fig. 2c).

Morphological and physiological trait links
CT\(_{\text{min}}\) best predicted morphological trait variation in all cases, though the improvement in model
fit between CT\(_{\text{min}}\) and CT\(_{\text{max}}\) on FL was slight (< 2 AIC), suggesting that both predicted FL
equally well. We retained CT\(_{\text{min}}\), as a predictor of FL because it was a slightly better fit, and it
was consistent with the IND and REL models. IND decreased significantly with CT\(_{\text{min}}\) (coef. = -
0.875, SE = 0.128, t-value = -6.862, p-value < 0.001), indicating that ants tolerating lower
minimum temperatures were darker in leg color and antenna (Fig. 3a). REL and FL increased
significantly with elevation: REL, coef. = 0.377, SE = 0.186, t-value = 2.011, p-value = 0.047;
FL, coef. = 0.028, SE = 0.015, t-value = 1.892, p-value = 0.061 (Fig. 3b, c). These results
indicate that ants tolerating lower minimum temperatures had smaller eyes relative to their head
size and shorter legs.

Geographical species trait differences
Both IND and REL differed significantly between A. picea specimens from CWT and A. rudis
specimens from WHF: IND, coef. = -1.262, SE = 0.467, z-value = -2.704, p-value = 0.007; REL,
coef. = 0.521, SE = 0.249, z-value = 2.087, p-value = 0.037 (Fig. 4a,b). These results indicate
that the A. picea specimens at CWT had darker leg and antenna color and smaller eyes relative to
their head size as compared to the *A. rudis* specimens from WHF. There was no difference in FL between specimens from CWT and WHF (*coef. = 0.006, SE = 0.005, z-value = 1.095, p-value = 0.273* (Fig. 4C).

**Discussion**

Crozier (1977) identified an ecotone in the Southern Appalachian mountains where low elevation *Aphaenogaster rudis* shift to high elevation *A. picea* populations – species he differentiated based on chromosome number and color. Warren II and Chick (2013) re-sampled his gradient and found that the *A. rudis/A. picea* ecotone had shifted ca. 200 m upwards to ca. 750 m elevation due to an increase in minimum temperatures, but they noted that mid-elevation ant morphology appeared muddled between the species, suggesting hybridization rather than replacement (but see Harr and Price 2014). In the current study, we found no evidence of genetic hybridization, however, and the morphological shift in leg and antenna coloring at ca. 750 m was consistent with species rather than gene replacement. However, color was highly plastic, and additional distinguishing traits were more dependent on environment than species as they were linked with physiological cold tolerance and environment. Overall then, we found *Aphaenogaster* spp. morphology highly plastic and somewhat indistinguishable at local scales, but clearly genetically distinct and morphologically differentiated at broader scales.

Ant species in the *rudis* complex are widespread in N.A., hard to morphologically differentiate and are distributed in a manner that appears linked with post-glacial climates (King et al. 2013; Lubertazzi 2012; Warren II and Bradford 2013; Warren II and Chick 2013; Warren II et al. 2011b). Based on morphology, Creighton (1950) identified six species/subspecies in the *rudis* complex (*A. miamiana, A. picea, A. rudis, A. fulva, A carolinensis* and *A. texana*) and
suggested that color was a poor trait for distinguishing between them. Crozier (1977) questioned whether there were more cryptic species hiding in the *rudis* complex, and used chromosomal and isozyme variation to distinguish light colored coastal plain (*A. rudis*) and dark colored montane (*A. picea*) species, but suggested that color differences were too slight to be useful. Umphrey (1996) examined morphometrics in the *rudis* complex based on species identified with cytogenetic and electrophoretic markers and proposed nine species (four previously undescribed) with geographic location and coloring included as identifying characteristics. Subsequent direct and indirect testing of temperature requirements among the species supported ecological differentiation among the six identified species, particularly *A. rudis* and *A. picea* (Warren II et al. 2011a; Warren II and Bradford 2013; Warren II and Chick 2013; Warren II et al. 2011b).

Our genetic analyses suggest, as did Umphrey (1996), that additional cryptic species may be hidden within the six identified species. The two *A. picea* clades that have darker legs and the last 4 antennal segments lighter in color correspond to the DNA data that separates them from other samples in the *A. rudis* clade. DNA evidence also points to four smaller *A. rudis* clades, but without strong support. Due to this lack of support, no new species have been described at this time (De Marco and Cognato in press). Although *A. carolinensis* and *A. miamiana* are within *A. rudis*, they are missing an intron in the CAD gene. Morphologically, there is a tremendous amount of variability within the *A. rudis* clade. Whereas *A. carolinensis* tends to be lighter in color than *A. rudis*, the femur length character was too variable to be reliable (De Marco and Cognato in press). *Aphaenogaster miamiana* can still be identified by overall coarse sculpturing and inward pointing spines (Creighton 1950).

We found that antenna and leg coloring best distinguished between what we identified as *A. rudis* and *A. picea* at local (NGA gradient) and broad (CWT vs. WHF) scales, but with a
moderate degree of confidence. The relative eye index differentiated between the two species at the broad scale, but was not useful at the local scale. Femur length was not useful at either scale. We found that head width was relatively consistent in differentiating A. rudis and A. picea, but we also noted considerable variance between colonies within each study. Eye length and femur length were not useful at all in distinguishing the species. Umphrey (1996) noted that most rudis complex worker characters exhibit too much variance to be reliable for species identification, but suggested that slight size and shape differences were distinguishable, and color – particularly at broad scales – can be useful.

The overlap in morphology between species is explained by the linkage between the color, size and shape traits with physiological temperature tolerance; the strongest link occurs between darker color and higher elevation. Darker color (melanization) with higher altitude and latitude is a common inter- and intraspecific difference for many species as an adaptation for cold tolerance (Ellers and Boggs 2004; MontBlanc et al. 2007; Parkash and Munjal 1999; Robinson 2001). The blurring in coloring between A. rudis and A. picea at mid elevations suggests phenotypic plasticity in melanization so that they converge in similar environments. Additional variance in these trait values likely arises from within the elevation gradients due to striking temperature differences because of topographical heterogeneity, such as north- and south-facing slope aspect (Cantlon 1953; Warren II 2010).

Both the genetic work of Crozier (1977) and the results presented here show no evidence of hybridizing between sympatric A. rudis and A. picea populations. As such, the movement of A. rudis colonies upward appears to be replacing A. picea colonies. Whether structured by competition or habitat selection, or some combination of both, high-elevation ant species were in fact, replaced by low-elevation congeners with no evidence of interbreeding.
Acknowledgements

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texana complex of the ant genus *Aphaenogaster* (Hymenoptera: Formicidae) Can J Zool
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differences create no-analogue communities and cause extinctions during climate change
Climate-driven range shift


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Climate-driven range shift

### Table 1 – *Aphaenogaster* and outgroup specimens with associated localities and Genbank numbers

<table>
<thead>
<tr>
<th>Taxon name, author and specimen number</th>
<th>Collection locations</th>
<th>GPS coordinates</th>
<th>Elevation (meters)</th>
<th>Genbank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. carolinensis Wheeler 2 NC</em></td>
<td>USA, North Carolina</td>
<td>45.29 W, 78.17</td>
<td>35.364</td>
<td>EF186427, EF186354, EF183029</td>
</tr>
<tr>
<td><em>A. carolinensis 3 NC</em></td>
<td>USA, North Carolina</td>
<td>28.18 W, 79.47</td>
<td>4.648</td>
<td>EF186428, EF186355, EF183030</td>
</tr>
<tr>
<td><em>A. carolinensis 15 NC</em></td>
<td>USA, North Carolina</td>
<td>28.18 W, 79.47</td>
<td>4.648</td>
<td>EF186427, EF186354, EF183029</td>
</tr>
<tr>
<td><em>A. carolinensis 16 MS</em></td>
<td>USA, Mississippi</td>
<td>30.88 W, 89.43</td>
<td>44.353</td>
<td>EF186428, EF186355, EF183030</td>
</tr>
<tr>
<td><em>A. fulva 1VA</em></td>
<td>VA, Surry Co.</td>
<td>24.59 W, 76.42</td>
<td>4.648</td>
<td>EF186427, EF186354, EF183029</td>
</tr>
<tr>
<td><em>A. fulva 6 NC</em></td>
<td>USA, North Carolina</td>
<td>46.50 W, 78.17</td>
<td>39.480</td>
<td>EF186428, EF186355, EF183030</td>
</tr>
<tr>
<td><em>A. fulva 10 CA</em></td>
<td>USA, Georgia</td>
<td>39.56 W, 89.43</td>
<td>52.718</td>
<td>EF186428, EF186355, EF183030</td>
</tr>
<tr>
<td><em>A. nigrocylindrus Wheeler 1 FL</em></td>
<td>USA, Florida</td>
<td>32.73 W, 89.43</td>
<td>7.35</td>
<td>EF186427, EF186354, EF183029</td>
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<tr>
<td><em>A. nigrocylindrus 5 NC</em></td>
<td>USA, North Carolina</td>
<td>42.50 W, 89.43</td>
<td>36.458</td>
<td>EF186428, EF186355, EF183030</td>
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</tr>
<tr>
<td><em>A. nigrocylindrus 2 MI</em></td>
<td>USA, Michigan</td>
<td>48.37 W, 89.43</td>
<td>22.376</td>
<td>EF186428, EF186355, EF183030</td>
</tr>
<tr>
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A. = *Aphaenogaster*
Fig. 1 – Principal component analysis of ant morphological traits (all caps; EW = eye width, EL = eye length, HL = head length, HW = head width, FL = femur length), morphological indices (all caps; ATN = antenna, LEG = leg), physiological tolerance (CT$\text{max}$ = maximum thermal tolerance, CT$\text{min}$ = minimum thermal tolerance), local climate (T$\text{max}$ = maximum temperature, T$\text{min}$ = minimum temperature, Prec = precipitation) and elevation (Elev). Arrows pointing in the same direction indicate positive covariation and those pointing in opposite directions indicate negative covariation.
Fig. 2 – Changes in *Aphaenogaster* ant leg/antenna color index (a), the relative eye index (b) and femur length (c) along elevation gradients. The fitted line includes both species, and the legend indicates the residual contribution of *A. picea* and *A. rudis*.
**Fig. 3**—Changes in *Aphaenogaster* ant leg/antenna color index (a), the relative eye index (b) and femur length (c) with minimum temperature (CT$_{\text{min}}$). The fitted line includes both species, and the legend indicates the residual contribution of *A. picea* and *A. rudis*. 
Fig. 4 – Differences in the leg/antenna color index (a), the relative eye index (b) and femur length (c) trait values for *Aphaenogaster picea* at the Coweeta Hydrologic Laboratory (U.S.) and *A. rudis* at Whitehall Forest (U.S.).
S1 – Bayesian majority rule consensus tree reconstructed for 52 taxa with morphology and five genes in a Mr. Bayes analysis. Posterior probabilities values greater than 90% are above the branches (* > 90%, **= 100%). *A*. = *Aphaenogaster*. Specimen numbers and states/provinces where collected are displayed next to each sample. The names of non-monophyletic species correspond to specific colors.