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

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Abstract

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; Hairston 1949; Hairston 1951; Hairston et al. 1992; Highton and Peabody 2000).

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).

101 The ant samples were dried and stored frozen at 4°C until 2013 when they were pinned and
102 mounted for morphometric analysis. A total of 162 specimens from 2011 and 54 specimens from
103 2012 were used. Voucher specimens are stored at the Georgia Museum of Natural History
104 (GMNH) and SUNY Buffalo State's insect collection. Location data and morphometric
105 measurements also were taken from voucher specimens collected by Crozier (1977) [CRO] and
106 stored at the GMNH.
107
108 *Morphometrics*
109 Morphometric measurements were taken using a Leica M125 stereomicroscope with DFC295
110 digital camera and Leica Application Suite V4. Five traits were measured: head width (HW,
111 maximum width of head including outer edges of eyes), head length (HL, maximum length of
112 head excluding mandibles), eye width (EW, maximum eye width), eye length (EL, maximum
113 eye length) and femur length (FL, maximum femur length, posterior view). Given that *A*. *rudis*
114 ant legs typically are lightly colored and reddish and *A*. *picea* legs darker black, and that *A*. *rudis*
115 antenna segments are of uniform color whereas the last four segments of *A*. *picea* antenna are
116 lighter (Ellison et al. 2012), color and antenna indices were generated. The leg color index (LEG)
117 was coded as 1 = light, 2 = medium, 3 = dark; the antenna index (ATN) was coded as 1 =
118 segments uniform, 2 = last four segments somewhat lighter than rest, 3 = last four segments
119 clearly lighter than rest. Two technicians not involved in Warren II and Chick (2013)
120 independently coded index specimens.
121
122 *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_{max}) and minimum (T_{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). For ant thermal tolerance, we used the minimum (CT_{min}) and maximum (CT_{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 for10 individuals (5 for CT_{min}, 5 for CT_{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 "prncomp" 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_{min} and CT_{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

191 The phylogenetic analysis produced a mostly resolved Bayesian tree with strong support for the
192 outgroups, including *Camponotus pennsylvanicus*, *Formica glacialis*, *Stenamma diecki*,
193 *Novomessor cockerelli*, *Veromessor julianus* and *Solenopsis invicta* (S1). There also was strong
194 support for the *A. fulva* and *A. picea* clades, however *A. picea* was separated into two smaller
195 clades. The sample from North Carolina was in the smaller clade. *Aphaenogaster carolinensis*
196 and *A. miamiana* clades each showed strong support, however they were within the less
197 supported *A. rudis* clade. *Aphaenogaster carolinensis* and *A. miamiana* were distinguished from
198 *A. rudis* due to a missing intron in the gene CAD.
199
200 *Principal component analysis*
201 Principal component analysis on the full set of ant physical and physiological traits, climate
202 variables and elevation indicated that six principal components accounted for 96% of the
203 variance in the 13 variables. We plotted the first two components, which accounted for 74% of
204 the variance (Fig. 1). Prec and Elev were interchangeable, and they were negatively correlated
205 with T_{max}, CT_{min}, CT_{max} and FL along the PC1 axis (which accounted for 60% of the variability).
206 EL, HW, HL and EW appeared to covary together, particularly HW and HL, which were
207 interchangeable, and they were orthogonal to the PC1 axis, oriented more toward the PC2 axis
208 (which accounted for 14% of the variability). ATN and LEG covaried together, were oriented
209 toward the PC2 axis, and were negatively correlated with T_{min}.
210
211 *Trait variation with elevation*
212 IND (leg/antenna coloring index) increased significantly with elevation (*coef.* = 0.006, *SE* =
213 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_{min} best predicted morphological trait variation in all cases, though the improvement in model fit between CT_{min} and CT_{max} on FL was slight (< 2 AIC), suggesting that both predicted FL equally well. We retained $CT_{min,}$ 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_{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

237 their head size as compared to the *A. rudis* specimens from WHF. There was no difference in FL
238 between specimens from CWT and WHF (*coef.* = 0.006, *SE* = 0.005, *z-value* = 1.095, *p-value* =
239 0.273 (Fig. 4C).
240

241 **Discussion**

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

255 Ant species in the *rudis* complex are widespread in N.A., hard to morphologically
256 differentiate and are distributed in a manner that appears linked with post-glacial climates (King
257 et al. 2013; Lubertazzi 2012; Warren II and Bradford 2013; Warren II and Chick 2013; Warren II
258 et al. 2011b). Based on morphology, Creighton (1950) identified six species/subspecies in the
259 *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.

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

463 numbers

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

Taxon name, author and specimen number	Collection locations	GPS coordinates			Elevation meters	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
A. carolinenesis Wheeler 2 NC	USA: North Carolina	N34	45.529 W78	35.384	25	KP730069	KP860427	KP730151	KP860354	KP730229
A. carolinenesis 3 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730070	KP860428	KP730152	KP860355	KP730230
A. carolinenesis 12 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730071	KP860429	KP730153	KP860356	KP730231
A. carolinenesis 16 MS	USA: Mississippi	N33	30.800 W88	44.133	91	KP730072	KP860430	KP730154	KP860357	KP730232
A. fulva 1VA	VA: Surry Co.	N37	24.595 W76	42.767	17	KP730074	KP860431	KP730156	KP860359	KP730234
A. fulva 6 NC	USA: North Carolina	N35	46.560 W78	39.480	93	KP730076	KP860433	KP730158	KP860361	KP730236
A. fulva 18 GA	USA: Georgia	N34	39.961 W83	52.718	513	KP730082	KP860439	KP730164	KP860367	KP730242
A. miamiana Wheeler 1FL	USA: Florida	N32	32.733 W82	7.35	1068	KJ920531	KJ920563	KJ920595	KJ920631	KJ920666
A. miamiana 2 FL	USA: Florida	N32	32.733 W82	7.35	1068	KP730086	KP860443	KP730168	KP860371	KP730246
A. miamiana 3 FL	USA: Florida	N32	32.733 W82	7.35	1068	KP730087	KP860444	KP730169	KP860372	KP730247
A. miamiana 5 NC	USA: North Carolina	N34	42.502 W78	36.038	18	KP730088	KP860445	KP730170	KP860373	KP730248
A. picea (Wheeler) 1MI	USA: Michigan	N42	42.725 W84	22.392	258	KJ920535	KJ920565	KJ920599	KJ920635	KJ920670
A. picea 2 MI	USA: Michigan	N42	48.373 W84	22.376	258	KP730091	KP860448	KP730173	KP860376	KP730251
A. picea 3 OH	USA: Ohio	N41	27.505 W81	49.252	16	KP730092	KP860449	KP730174	KP860377	KP730252
A. picea 4 NY	USA: New York	N40	50.612 W73	10.350	16	KP730093	KP860450	KP730175	KP860378	KP730253
A. picea 15 MI	USA: Michigan	N42	47.807 W84	22.885	258	KP730094	KP860451	KP730176	KP860379	KP730254
Apicea 19 MA	USA: Massachusetts	N42	22.789 W71	9.231	11	KP730096	KP860453	KP730178	KP860381	KP730256
A. picea 21 MA	USA: Massachusetts	N42	32.203 W72	11.235	358	KP730097	KP860454	KP730179	KP860382	KP730257
Apicea 23 MI	USA: Michigan	N42	42.851 W84	22.404	258	KP730098	KP860455	KP730180	KP860383	KP730258
A. picea 26 NC	USA: North Carolina	N35	30.49 W82	53.59	1008	KP730099	KP860456	KP730181	KP860384	KP730259
A. picea 27 VA	USA:Virginia	N38	5.800 W78	46.8	685	KP730100	KP860457	KP730182	KP860385	KP730260
A. picea 28 MA	USA: Massachusetts	N42	16.13 W72	37.54	146	KP730101	KP860458	KP730183	KP860386	KP730261
A. picea 36 MA	USA: Massachusetts	N42	46.38 W70	51.599	2	KP730105	KP860462	KP730187	KP860390	KP730265
A. picea 37 MA	USA: Massachusetts	N41	31.319 W71	1.5	8	KP730106	KP860463	KP730188	KP860391	KP730266
A. picea 39 MA	USA: Massachusetts	N42	16.13 W72	37.54	146	KJ920537	KJ920567	KJ920601	N/A	KJ920672
A. rudis Enzmann 2 MI	USA: Michigan	N44	44.555 W84	38.013	359	KJ920538	KJ920568	KJ920602	KJ920637	KJ920673
A. rudis 4 OH	USA: Ohio	N41	28.325 W81	51.623	16	KJ920539	KJ920569	KJ920603	KJ920638	KJ920674
A. rudis 6 VA	USA: Virginia	N38	3.233 W78	28.983	147	KP730107	KP860464	KP730189	KP860392	KP730267
A. rudis 8 NJ	USA: New Jersey	N40	29.000 W74	25.583	19	KJ920540	KJ920570	KJ920604	KJ920639	KJ920675
A. rudis 10 MN	USA: Minnesota	N44	0.417 W92	27.85	304	KP730108	KP860465	KP730190	KP860393	N/A
A. rudis 12 NJ	USA: New Jersey	N40	26.233 W74	15.533	4	KP730109	KP860466	KP730191	KP860394	KP730268
A. rudis 15 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730111	KP860468	KP730193	KP860396	N/A
A. rudis 16 OH	USA: Ohio	N39	34.165 W82	2.000	232	KP730112	KP860469	KP730194	KP860397	KP730270
A. rudis 17 NC	USA: North Carolina	N35	23.54 W83	24.46	1167	KP730113	KP860470	KP730195	N/A	KP730271
A. rudis 19 VA	USA:Virginia	N37	24.567 W76	42.817	17	KP730114	KP860471	KP730196	KP860398	KP730272
A. rudis 28 MA	USA: Massachusetts	N42	18.354 W72	28.431	153	KP730115	KP860472	KP730197	KP860399	KP730273
A. rudis 32 GA	USA: Georgia	N34	39.961 W83	52.718	518	KP730117	KP860474	N/A	KP860401	KP730275
A. rudis 33 GA	USA: Georgia	N34	54.299 W83	24.822	2057	KP730118	KP860475	N/A	KP860402	KP730276
A. rudis 34 PA	USA: Pennsylvania	N40	16.971 W79	10.626	413	KP730119	KP860476	KP730199	KP860403	N/A
A. rudis 41 GA	USA: Georgia	N34	52.746 W83	34.739	1734	KP730120	KP860477	KP730200	KP860404	KP730277
A. rudis 46 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730124	KP860481	KP730204	KP860407	KP730280
A. rudis 47 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730125	KP860482	KP730205	KP860408	KP730281
A. rudis 48 NC	USA: North Carolina	N36	2.186 W79	4.648	177	KP730126	KP860483	KP730206	KP860409	KP730282
A. rudis 49 NC	USA: Virginia	N38	18.717 W77	38.833	102	KP730127	KP860484	KP730207	KP860410	KP730283
A. rudis 50 NC	USA: Georgia	N34	42.343 W83	23.328	1478	KP730128	KP860485	KP730208	KP860411	KP730284
A. rudis 51 GA	USA: Georgia	N34	42.343 W83	23.328	1478	KP730129	KP860486	KP730209	KP860412	KP730285
Camponotus pennsylvanicus (DeGeer)	USA: Michigan	N42	42.878 W84	22.278	272	KJ920508	N/A	KJ920573	KJ920610	KJ920644
Formica glacialis Wheeler	USA: Michigan	N42	27.185 W84	1.019	295	KJ920509	N/A	KJ920574	KJ920611	KJ920645
Novomessor cockerelli André 1	USA: Arizona	N31	57.950 W109	9.300	1402	KJ920520	KJ920553	KJ920585	KJ920620	KJ920655
Solenopsis invicta Buren	USA: Mississippi	N31	57.766 W109	9.228	1400	KP730067	KP860425	KP730149	N/A	KP730228
Stenamma diecki Emery	USA: Minnesota	N33	16.368 W88	49.997	72	JQ742647	JQ742591	JQ742693	JQ742738	JQ742903
Veromessor julianus (Pergande)	Mexico: Baja CA Sur	N37	13.86 W122	6.512	880	KJ920511	KJ920546	KJ920575	KJ920612	KJ920646

A. = *Aphaenogaster*

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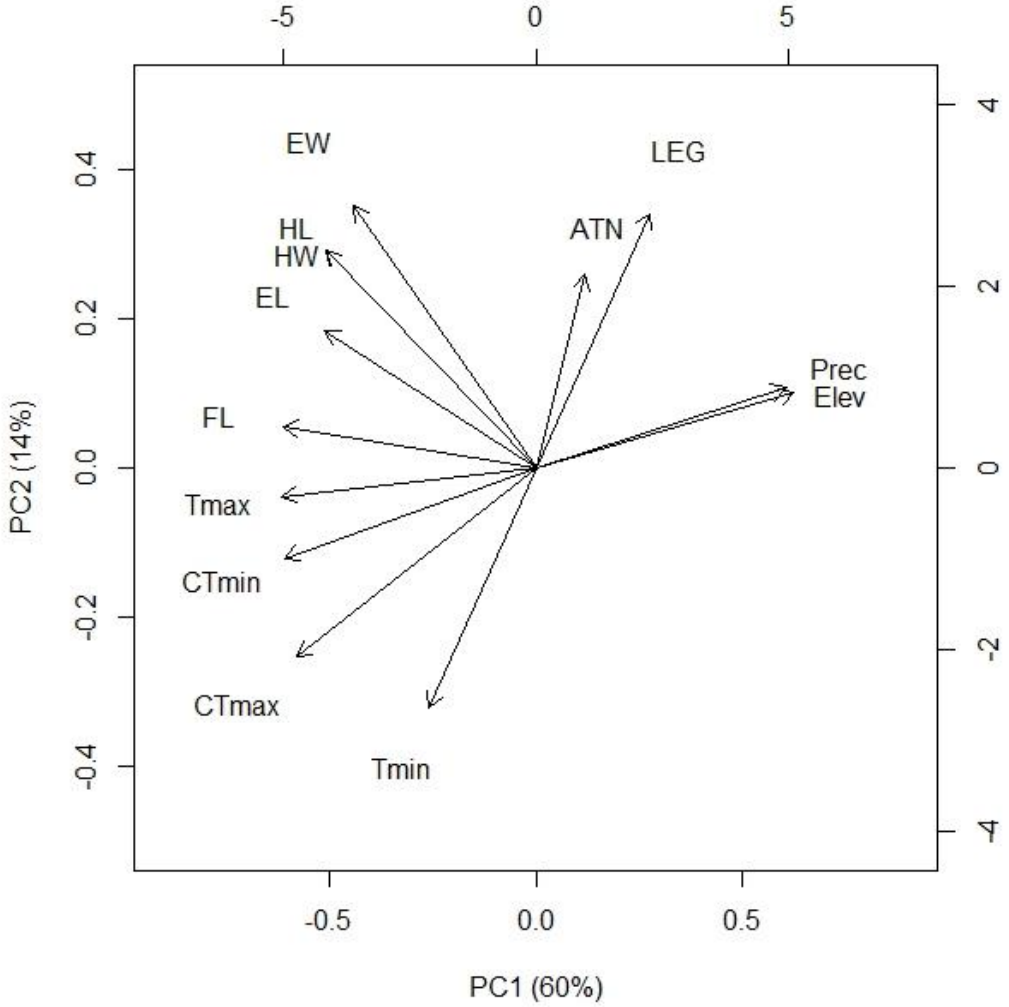
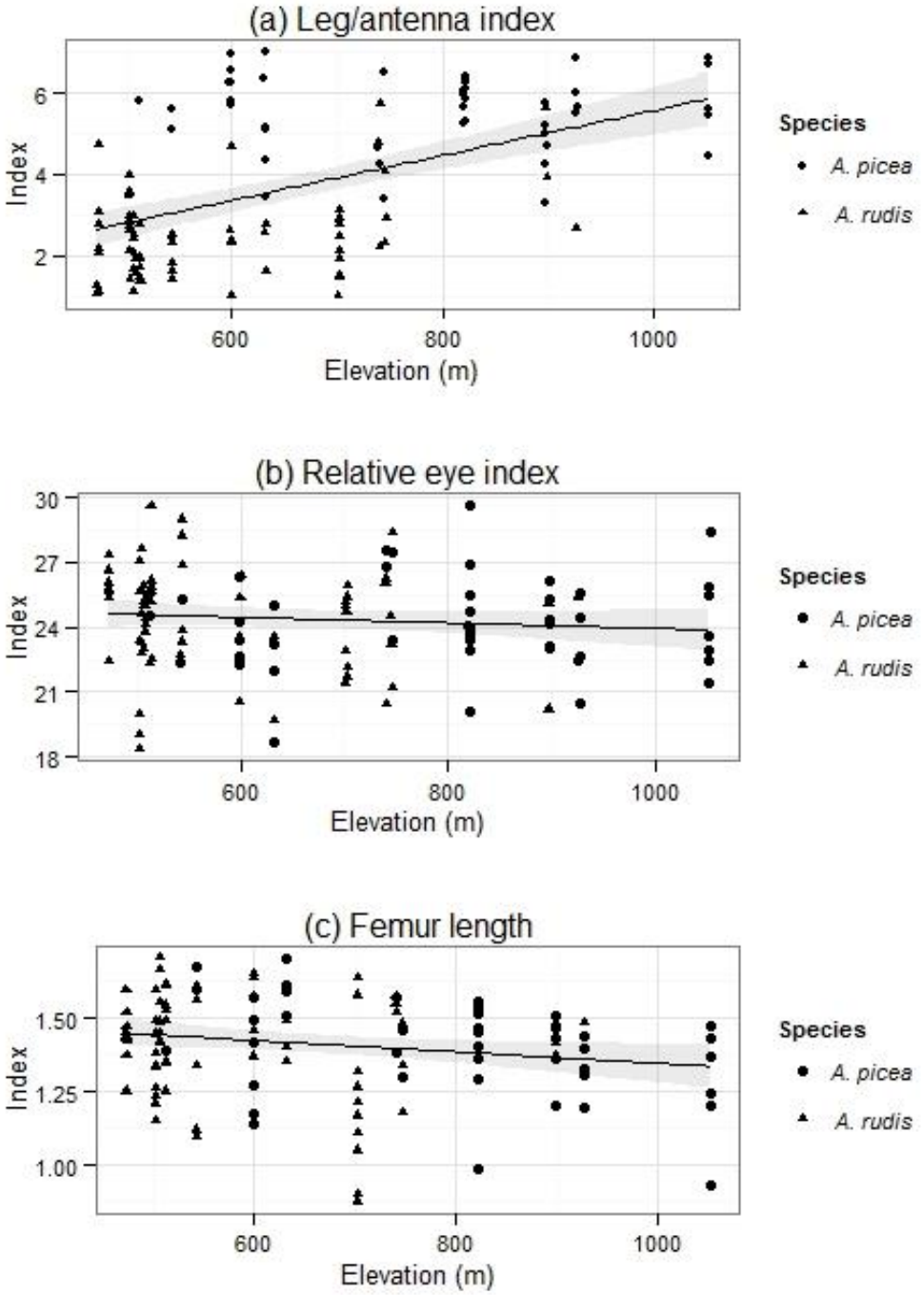


Fig. 1 – Principal component analysis of ant morphological traits (all caps; EW = eye width, EL = eye length, HL = head length, HW = head width, FL = femur length), morphological indices (all caps; ATN = antenna, LEG = leg), physiological tolerance (CT_{max} = maximum thermal tolerance, CT_{min} = minimum thermal tolerance), local climate (T_{max} = maximum temperature, Tmin = 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.



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477 **Fig. 2** – Changes in *Aphaenogaster* ant leg/antenna color index (a), the relative eye index (b) and

478 femur length (c) along elevation gradients. The fitted line includes both species, and the legend

479 indicates the residual contribution of *A*. *picea* and *A*. *rudis*.

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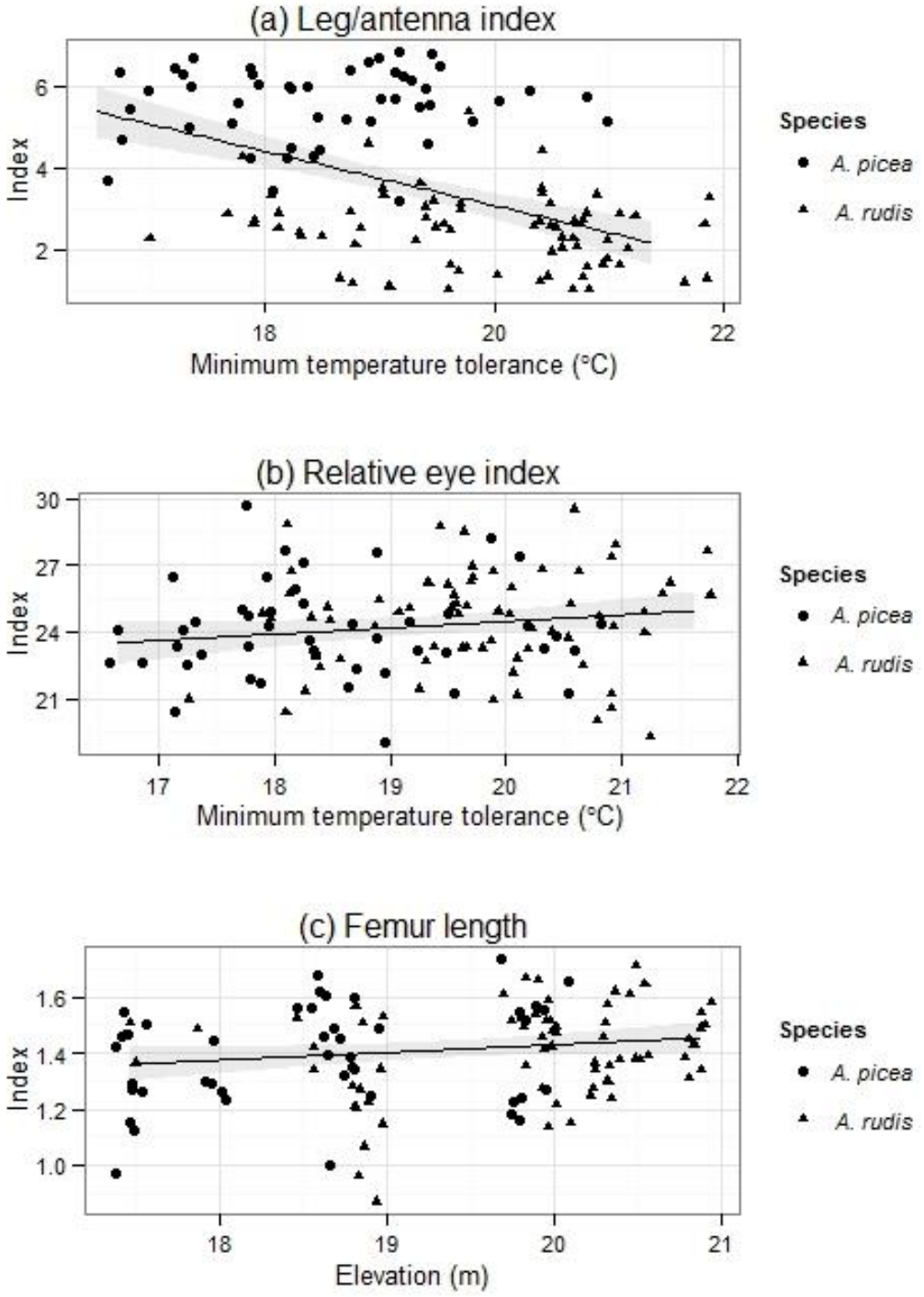
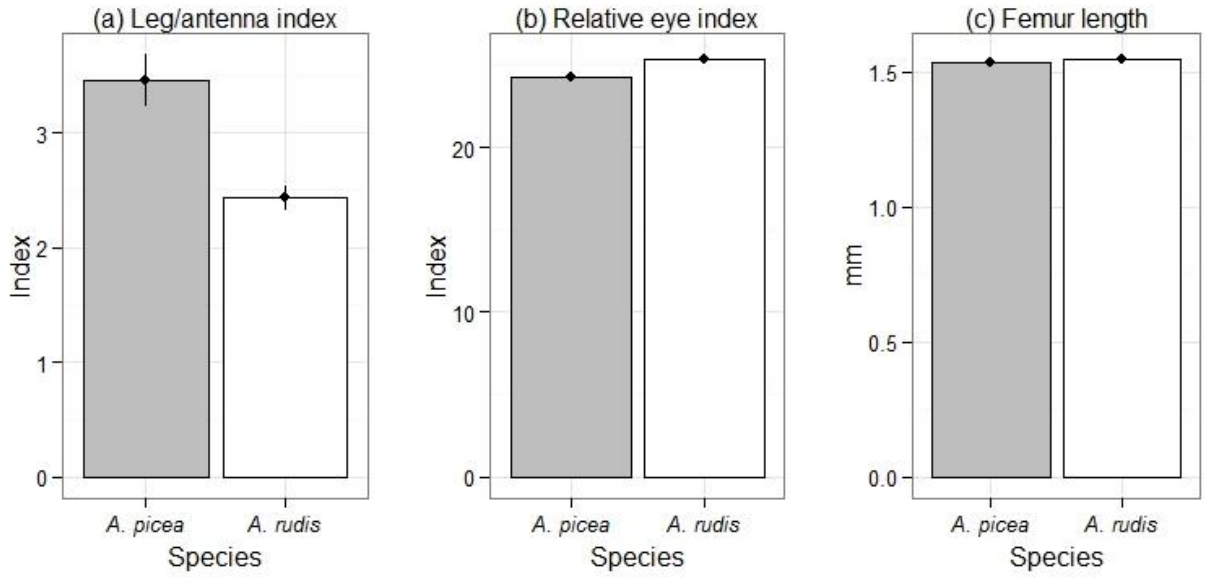


Fig. 3 –Changes in *Aphaenogaster* ant leg/antenna color index (a), the relative eye index (b) and femur length (c) with minimum temperature (CT_{min}). The fitted line includes both species, and the legend indicates the residual contribution of *A. picea* and *A. rudis*.



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486 **Fig. 4** – Differences in the leg/antenna color index (a), the relative eye index (b) and femur

487 length (c) trait values for *Aphaenogaster picea* at the Coweeta Hydrologic Laboratory (U.S.) and

488 *A. rudis* at Whitehall Forest (U.S.).

489

490

491 **S1** – Bayesian majority rule consensus tree reconstructed for 52 taxa with morphology and five
492 genes in a Mr. Bayes analysis. Posterior probabilities values greater than 90% are above the
493 branches (* > 90%, **= 100%). *A*. = *Aphaenogaster*. Specimen numbers and states/provinces
494 where collected are displayed next to each sample. The names of non-monophyletic species
495 correspond to specific colors.
496