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RESEARCH ARTICLE

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Evolutionary history, not ecogeographic rules, explains size variation of tropical insects along elevational gradients

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Abstract

1. One of the best-known biogeographic rules for ectotherms is the temperature-size rule, which asserts that ectotherms produce smaller adults at warmer temperatures. Although this is often true, it has become clear that there is no single process behind the pattern and many exceptions to the rule. To disentangle such complex temperature–size relationships, individual clades must be examined at ecological and evolutionary scales.
2. We examined temperature–size relationships for 2,106 individuals from 64 populations and 40 species of *Cephaloleia* rolled-leaf beetles (Chrysomelidae; Cassidinae) occurring along two tropical elevational gradients: Barva and the Talamanca Cordillera in Costa Rica, Central America. We tested whether the temperature-size rule applied to interspecific elevational assemblages, intraspecific elevational populations or different rearing temperatures for individual populations.
3. At the interspecific scale, evolutionary history, rather than elevation, explains body size. At the intraspecific scale, only one of seven species followed the temperature–size rule across elevations. When larvae were reared at different temperatures, only one of five populations followed the temperature–size rule. Most populations grew to a fixed size regardless of temperature.
4. Size in *Cephaloleia* beetles is constrained by their evolutionary history and responds to factors that rarely correlate with temperature. As temperature increases, ectotherms will not universally shrink, but determining if and why their size will change will require further investigation.

KEYWORDS
Bergmann's rule, developmental plasticity, neotropics, rolled-leaf beetles

1 | INTRODUCTION

Variation in body size among populations and species has stimulated both empirical and theoretical predictions on how size may change with temperature, elevation and latitude (Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011). From ecological and physiological perspectives, body size also represents biomass, which determines both the amount of resources that must be consumed and the resources available to potential consumers (Verberk, Buchwalter, & Kefford, 2020). Changes in body size affect fecundity, lifespan, population dynamics and species composition (Daufresne, Lengfellner, & Sommer, 2009). How temperature affects body size has implications beyond theoretical issues. As humans alter temperature regimes through global warming, changes in organisms' body sizes may affect ecological and evolutionary processes worldwide (Daufresne et al., 2009; Ohlberger, 2013).

Predictions on how temperature affects body size can be summarized by three ecogeographic rules. One of the oldest ecological

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generalizations, 'Bergmann's rule', compares body size across closely related species of endotherms, and predicts that warmer localities tend to be inhabited by small-sized species (Bergmann, 1847). This pattern is assumed to emerge from trade-offs between temperature and the thermoregulatory advantages associated with surface-area-to-volume ratios (Blanckenhorn & Demont, 2004; Gardner et al., 2011). Whether ectotherms should be considered under Bergmann's rule is still a topic of discussion (Blackburn, Gaston, & Loder, 1999; Daufresne et al., 2009; Lomolino, Riddle, & Brown, 2006).

A more broadly accepted hypothesis to explain correlations between size and temperature among species of ectotherms is the 'temperature-size rule', which states that body size tends to decrease with increasing temperature (Atkinson, 1994). The mechanism assumed to generate this pattern is a reduction in development time at higher temperatures. Although a reduction in generation time may accelerate population growth, it also reduces adult body size (Atkinson, 1994; García-Robledo & Horvitz, 2011). A third ecogeographic rule, 'James' rule' focuses on the relative effect of temperature on body size between populations. James' rule predicts a decrease in body size when populations experience warmer temperatures (James, 1970). Differences in body size among populations are attributed to the same processes assumed by Bergman's and the temperature–size rules (Gardner et al., 2011).

The relationship between body size and temperature is affected by different abiotic factors along latitudinal or elevational gradients. Temperature gradients are 1,000 times steeper in elevational than in latitudinal gradients (5.2–6.5°C per 1,000 m elevation vs. 6.9°C per 1,000 km at 45°N or S; Colwell, Brehm, Cardelus, Gilman, & Longino, 2008; Rohde et al., 2013). At increasing latitudes, organisms also experience an increase in seasonality (Janzen, 1967). Body size of temperate arthropods is modulated by plasticity in diapause, polyphenism and quiescence, and tends to increases with latitude (Anderson, Hoffmann, & McKechnie, 2005; Horne, Hirst, & Atkinson, 2015; Sgro, Terblanche, & Hoffmann, 2016). Most tropical arthropods are multivoltine and body size will depend on the plasticity of developmental traits such as larval growth rates and time to pupation (Shama, Campero-Paz, Wegner, De Block, & Stoks, 2011).

Oxygen partial pressure and air density also decrease with elevation (Dillon, Frazier, & Dudley, 2006). Under laboratory conditions, low oxygen pressure induces moulting and a reduction in body size in fruit flies and the tobacco hornworm (Callier & Nijhout, 2011; Peck & Maddrell, 2005). Physiologies of aquatic ectotherms are limited by low oxygen concentration, especially in standing waters (Verberk et al., 2020). Temperature–size relationships in aquatic ectotherms are modulated by oxygen availability and oxygen demand, and may have even a stronger effect on body size than temperature (Hoefnagel & Verberk, 2015). For terrestrial arthropods, there is no apparent association between a reduction of oxygen and body size, even along the highest elevational gradients on earth (Dillon et al., 2006).

All three ecogeographic rules predict that body size will decline with global warming (Gardner et al., 2011). However, exceptions are commonplace, showing that ecogeographic rules are more general tendencies rather than absolute natural laws (Lomolino et al., 2006). At least for ectotherms, one possibility for such exceptions are trade-offs between temperature, size and fitness (Kingsolver & Huey, 2008). These pressures can be summarized as 'bigger is better', 'hotter is better', but 'hotter is smaller'. In other words, while larger individuals have higher fitness than their smaller conspecifics and hotter temperatures lead to higher maximum fitness, hotter temperatures can also lead to smaller body size (Kingsolver & Huey, 2008). Given this contradiction, natural selection might favour developmental and physiological mechanisms that disrupt the negative correlation between temperature and body size (Kingsolver & Huey, 2008).

Determining how temperature affects body size is challenging because although all ecogeographic rules share the same prediction, each rule invokes different mechanisms. An additional complication is that to determine the role of each mechanism, experiments must also disentangle the relative effects of adaptation and plasticity on body size. This means that the questions of how and why body size responds to temperature are not two universal questions but a series of questions that must be addressed all ecological levels, from populations to species and community, and in a phylogenetic context.

Regardless of whether inter- or intraspecific size differences are of interest, the first question must always be 'what is the temperature-size relationship for the focal monophyletic group?' This can be answered by comparing natural populations at different temperatures, whether across latitudes, seasons or elevations. Tests for interspecific temperature–size relationships should use a phylogenetic approach to control for shared evolutionary history. Once the type of temperature–size relationship has been identified, the question 'what causes that temperature-size relationship?' should be asked. If an interspecific phylogenetic analysis shows that size differences are consistent with a Brownian model of evolution, the answer may be as simple as 'shared evolutionary history'. For intraspecific comparisons, rearing experiments can help determine whether body size differences (or similarities) result from plasticity or fixed genetic differences.

We addressed these questions using 64 populations from 40 species of rolled-leaf beetles (*Cephaloleia*, Family Chrysomelidae) distributed along two tropical elevational gradients in Costa Rica, Central America. In previous studies, we determined that rolled-leaf beetle species are adapted to temperatures prevalent in their habitats, with high-elevation species having lower thermal tolerance than species in the lowlands (García-Robledo, Kuprewicz, Staines, Erwin, & Kress, 2016). It is possible that body size of rolled-leaf beetles also reflects such adaptations to local temperatures.

In this study, we investigated the inter- and intraspecific relationships between environmental temperature and body size over both natural and laboratory temperature gradients. First, we asked whether *Cephaloleia* species from assemblages at a given elevational life zone (e.g. tropical lowland, premontane or montane forests) display similar size. For species that occurred at multiple elevations, we also asked whether there were differences in body size among

populations. Finally, we asked whether temperature differences alone could create intrapopulation differences in body size in five laboratory-reared beetle populations. In all scenarios, we hypothesized that *Cephaloleia* at higher temperatures would have smaller body sizes due to more rapid development, consistent with the ectotherm temperature–size and James' rules. Alternatively, using a phylogenetic approach, we tested if interspecific size differences can be attributed to evolutionary history and if individual species would have genetically determined sizes regardless of temperature.

2 | MATERIALS AND METHODS

2.1 | Study organisms and sites

Cephaloleia beetles (Chrysomelidae; Cassidinae) and plants from the order Zingiberales evolved in the neotropics for the last 40–60 MY (Garcia-Robledo & Staines, 2008; Gómez-Zurita, Hunt, Kopliku, & Vogler, 2007; Wilf et al., 2000). *Cephaloleia* are also known as the rolled-leaf beetles because larvae and adults feed inside the scroll formed by the young leaf of their host plants (Staines & García-Robledo, 2014). Rolled-leaf beetles are multivoltine, with larvae and adults of multiple generation inhabiting the same young leaves (García-Robledo, Horvitz, & Staines, 2010). The relative humidity inside rolled-leaf scrolls is always close to 100% (García-Robledo et al., 2010). Larvae breathe air, and may drown if fully covered with water (Johnson, 2004a, 2004b). We recorded elevational distributions of *Cephaloleia* species from 2005 to 2016. Beetles were collected on the Barva transect (eastern slope of the Central Cordillera 10°0′–10°26′N, 83°59′–84°07′W) and the western slope of the Talamanca Cordillera (8°38′–8°58′N, 82°50′–83°05′W). The two elevational gradients have similar monthly minimum temperatures, but monthly maximum temperatures in Talamanca are 1–3°C warmer (García-Robledo et al., 2016). On the Barva transect, beetles were collected at La Selva Biological Station (50 m a.s.l.) and transect shelters in Braulio Carrillo National Park (700, 1,070, 1,500 and 2,100 m a.s.l.). Collections on Talamanca were made between Ciudad Neily and Fila de Cal (60–400 m a.s.l.), as well as at Las Cruces Biological Station (1,200 m a.s.l.) and in La Amistad National Park (1,500–2,100 m a.s.l.). Beetles from these sites were assigned to three wet forest life zones based on elevation: lowlands (0–499 m), premontane (500–1,499 m) and montane (1,500–2,500 m; Clark, Hurtado, & Saatchi, 2015; Holdridge, 1947).

2.2 | Effects of elevation and evolutionary history on beetle body size

Cephaloleia beetles were identified using a combination of traditional taxonomy and DNA barcoding (see DNA extraction and amplification methods in references García-Robledo, Kuprewicz, Staines, Kress, & Erwin, 2013; Staines & García-Robledo, 2014). Several species display cytochrome oxidase 1 (*cox1*) haplotypes typical of each life zone (García-Robledo et al., 2016). We treated haplotypes with DNA barcode divergence higher than 10% as distinct experimental units. Previous studies showed that this degree of divergence is typical of well-delimited *Cephaloleia* species (García-Robledo et al., 2016). The specimens were measured individually using Moticam software (Motic) and a calibrated Fisher digital dissecting microscope.

We used these data to test associations between elevation and beetle size. To determine whether evolutionary history is associated with the life zone at which each species is present, or size, we performed a phylogenetic analysis using a *cox1* tree. We assembled the tree using one sequence per sampled population. We selected as outgroups two species of *Chelobasis* (Chrysomelidae), *Ch. bicolor* and *Ch. perplexa*. *Chelobasis* is a genus of rolled-leaf beetles specialized in plants from the family Heliconiaceae (Zingiberales; Staines, 2009). Sequences were aligned using MUSCLE (Edgar, 2004) and the tree was inferred using MrBayes (Ronquist & Huelsenbeck, 2003) on the CIPRES platform (Miller, Pfeiffer, & Schwartz, 2010). All subsequent analyses were performed in R Studio with R version 3.5.0 (RStudio Team, 2015; R Core Team, 2016).

We tested for phylogenetic signal in elevational gradient, life zone and size using Pagel's lambda (phytools version 0.6-99; Revell, 2012). This test uses a tree transformation from the initial topology, and assuming a Brownian motion evolution model, calculates the effect of gradually eliminating phylogenetic structure on the tree transformation parameter (phytools version 0.6-99; Revell, 2012). We then performed a phylogenetic least squares analysis of the relationship between size and the other variables (ape version 5.3; Paradis, Claude, & Strimmer, 2004). To determine whether there was intraspecific variation in size across life zones, we analysed the individual beetle lengths of species occurring in multiple life zones. Depending on whether a species was represented in two or three life zones, we performed either a *t* test or an ANOVA, respectively. Each ANOVA was followed by a Tukey's honest significant difference test to identify significant differences.

2.3 | Experiments testing the effects of temperature on body size

We performed these experiments at La Selva Biological Station, Costa Rica from September 2017 to November 2018. To test if temperature can directly influence adult size, we reared in the laboratory at multiple temperatures individuals of *C. belti* from lowland forests and *C. belti* from montane forests, as well as *C.* aff. *dilaticollis*, *C.* aff. *dorsalis* and *C. placida* from lowland forests. For each population, wild males and females were collected, paired, fed ad libitum with young leaf tissue from each species' most commonly used host plant, and given squares of mature leaf tissue for oviposition substrate (see host plant species in Figure 4). Every 48 hr, eggs were removed from the leaf tissue, individually placed in containers lined with filter paper, and distributed among environmental chambers built according to the specifications given in Garcia-Robledo, Kuprewicz, Dierick, Hurley, and Langevin (2020). At the beginning of the experiment, eggs were assigned to six

temperatures: 10, 15, 20, 25, 30 and 35°C. These temperatures range from the minimum temperature experienced in the montane forests to the predicted lowland maximum temperature in 2100 (Colwell et al., 2008). However, we discontinued some treatments as eggs in 10 and 35°C failed to hatch and all larvae in 30°C died before pupation. All lowland populations except *C. placida* were also unable to reach adulthood at 15°C and the montane *C. belti* were unable to do so at 25°C. Individuals in all treatments experienced a 12 hr–12 hr light–dark regime and were fed every 48 hr with discs of leaf tissue. We systematically rotated individuals within each environmental chamber to control for microclimatic differences within the chambers.

We measured adults the day after eclosion by photographing them with a digital camera (Model 3.2.0; Diagnostic Instruments, Inc.) attached to a stereoscope (Leica MZ 12s). We then estimated adult lengths from the photographs to the nearest 10^{-2} mm using the program SPOT V.3.5.8 (Diagnostic Instruments, Inc.). The effect of temperature on adult length for each species was tested using a *t* test or ANOVA depending on the number of treatments.

3 | RESULTS

3.1 | Differences in beetle assemblage body size across life zones

To determine whether beetle assemblages from different life zones differed in size, we measured 1,311 specimens. Interspecific comparisons also included 795 measurements previously included in a monograph of the genus (Staines & García-Robledo, 2014; see sample sizes in Supporting Information S1). The topology of the phylogenetic tree we reconstructed is similar to previous trees for portions of the genus (García-Robledo et al., 2016; McKenna & Farrell, 2005), although our more intensive sampling revealed that some taxonomic species contain widely separately mitochondrial lineages (Figure 1). When beetle traits were placed in the context of the phylogenetic tree, we found that size (Pagel's $\lambda = 0.999$; $p = 1.21 \times 10^{-19}$) displayed significant phylogenetic signal, but life zone (Pagel's $\lambda = 6.61 \times 10^{-5}$; $p = 1$) and elevational gradient did not (Pagel's $\lambda = 7.82 \times 10^{-5}$; $p = 1$). When the effect of shared evolutionary history was controlled for in the phylogenetic least-squares analysis, we found that neither of the variables considered (life zone or elevational gradient) explained beetle size (Table 1). Body size of beetle assemblages at different elevations is similar (Table 1; Figure 2).

TABLE 1 Phylogenetic least squares analysis of the relationship between beetle size, elevational gradient and life zone after controlling for shared evolutionary history

Variable	*F* statistic	*df*	*p*
Life zone	0.289	2, 58	0.750
Elevational gradient	1.220	1, 58	0.274
Life zone × gradient	0.150	2, 58	0.861

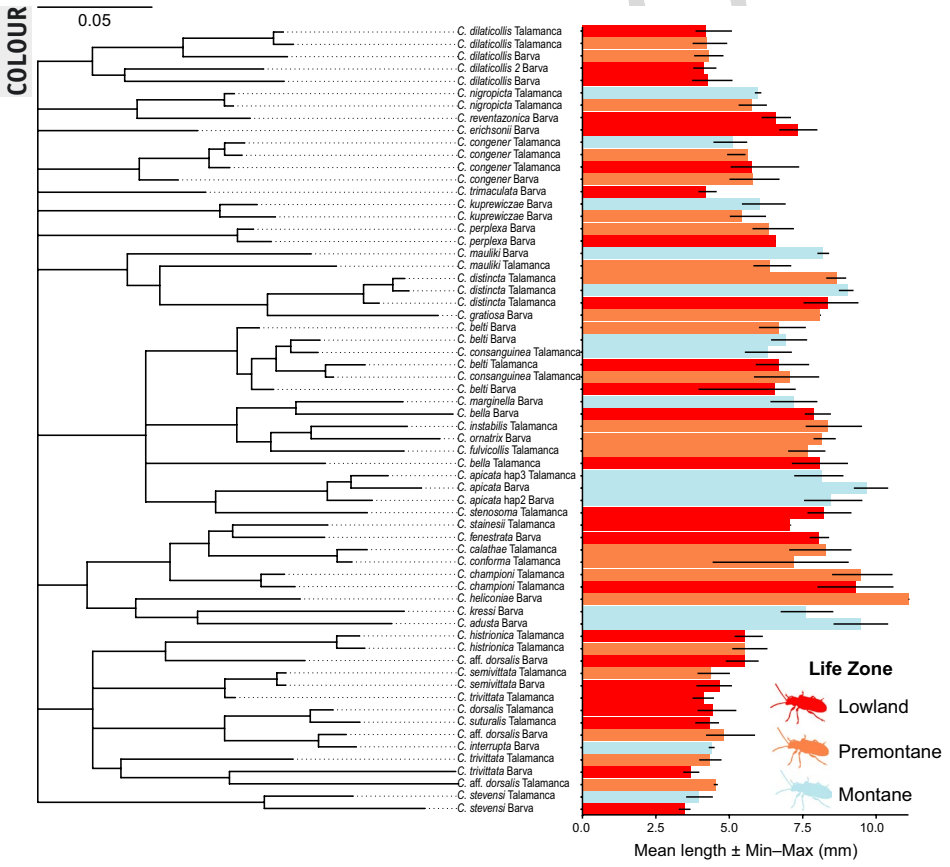

FIGURE 1 Phylogeny of Costa Rican *Cephaloleia* populations and their body sizes based on the DNA barcode *cox1*. Each tip represents a population found at a particular life zone on one of the elevational gradients. Bars represent minimum and maximum body lengths for each species and population. GenBank accession nos. KU357054–KU358485. Total number of individuals measured = 2,106, see sample sizes in Supporting Information S1

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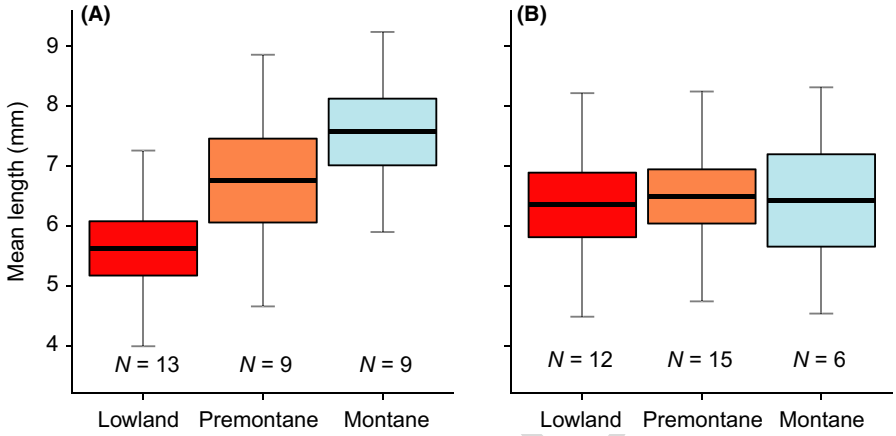

FIGURE 2 Lengths ($M \pm SE \pm SD$) of *Cephaloleia* beetle assemblages in different life zones along elevational gradients. (A) Barva transect *Cephaloleia* assemblages. (B) Talamanca *Cephaloleia* assemblages. Sample size in Supporting Information S1

TABLE 2 ANOVAs and *t* tests for intraspecific size variation by life zone

4

Species	Test statistic	*df*	*p*
Cephaloleia belti (Barva)	$F = 32.13$	2, 151	$\mathbf{2.4 \times 10^{-12}}$
Cephaloleia belti (Talamanca)	$F = 22.19$	2, 182	$\mathbf{2.4 \times 10^{-9}}$
Cephaloleia congener (Talamanca)	$F = 11.85$	2, 78	$\mathbf{3.2 \times 10^{-5}}$
Cephaloleia aff. *dilaticollis* (Talamanca)	$t = -0.851$	59.218	0.40
Cephaloleia championi (Talamanca)	$t = -0.661$	58.285	0.51
Cephaloleia histronica (Talamanca)	$t = -0.024$	29.744	0.98
Cephaloleia aff. *dilaticollis* (Barva)	$t = -0.644$	80.481	0.52

3.2 | Intraspecific differences in mean body length across multiple life zones

To determine whether elevation causes intraspecific variation in size, we compared mean body lengths between beetle populations in different life zones. In three cases, we were able to compare lowland, premontane and montane populations. In another four cases, we compared lowland and premontane populations.

We detected differences in mean body size between life zones in three cases (Table 2). In the Barva elevational gradient, *C. belti* body lengths increased with elevation (Figure 3A). In the Talamanca elevational gradient, lowland *C. belti* displays reduced body size, which increases at intermediate elevations, and becomes even smaller at the highest elevation (Figure 3D). For *C. congener* on Talamanca, body size decreases with elevation (Figure 3F). None of the species with populations in two life zones showed significant differences in size between lowland and premontane life zones (Figure 3; Table 2).

3.3 | Beetles reared at various temperatures

To determine whether size variation is caused by temperature alone, we reared 957 beetles from five Barva populations at constant temperatures in the laboratory. For lowland *C. belti*, there was no significant difference in length between adults reared in 20 and 25°C (Figure 4A; Table 3). Similarly, montane *C. belti* did not differ in length between 15 and 20°C (Figure 4B). As a group, however, reared lowland *C. belti* were significantly smaller than montane *C. belti* (6.114 mm vs. 6.441 mm, $t = -6.995$, $df = 126.52$, $p = 1.37 \times 10^{-10}$). For lowland *C.* aff. *dilaticollis*, adults raised at 20°C were significantly larger than 25°C adults (Figure 4C). For lowland *C.* aff. *dorsalis*, there was no significant difference between 20 and 25°C adults (Figure 4D). *Cephaloleia placida* was the only lowland population that was successfully reared at 15°C as well as 20 and 25°C and temperature affected adult size (Figure 4E). The 20°C *C. placida* were significantly larger than the 25°C adults (Tukey test $p = 0.00013$), but the 15°C adults had an intermediate length.

4 | DISCUSSION

We found that elevation and temperature affect beetle size, but the relationship is much more complex than the predictions of ecogeographic rules, or the axiom 'hotter is smaller'. Our hypothesis that whole *Cephaloleia* assemblages would increase in size with increasing elevation was rejected. Instead, the alternative hypothesis that beetle size was primarily determined by evolutionary history was supported. While there was no evidence for an effect of elevation on body size above the species level, in some cases elevation was associated with differences in intraspecific body size. Our hypothesis that conspecifics at higher elevations would be larger was only supported for one species, and along a particular elevational gradient. However, populations of species distributed along all three life zones show some variation in size. Our final hypothesis that beetles reared at different temperatures would exhibit size plasticity was supported for some but not all populations, and our results show that the relationship between temperature and size is species-dependent.

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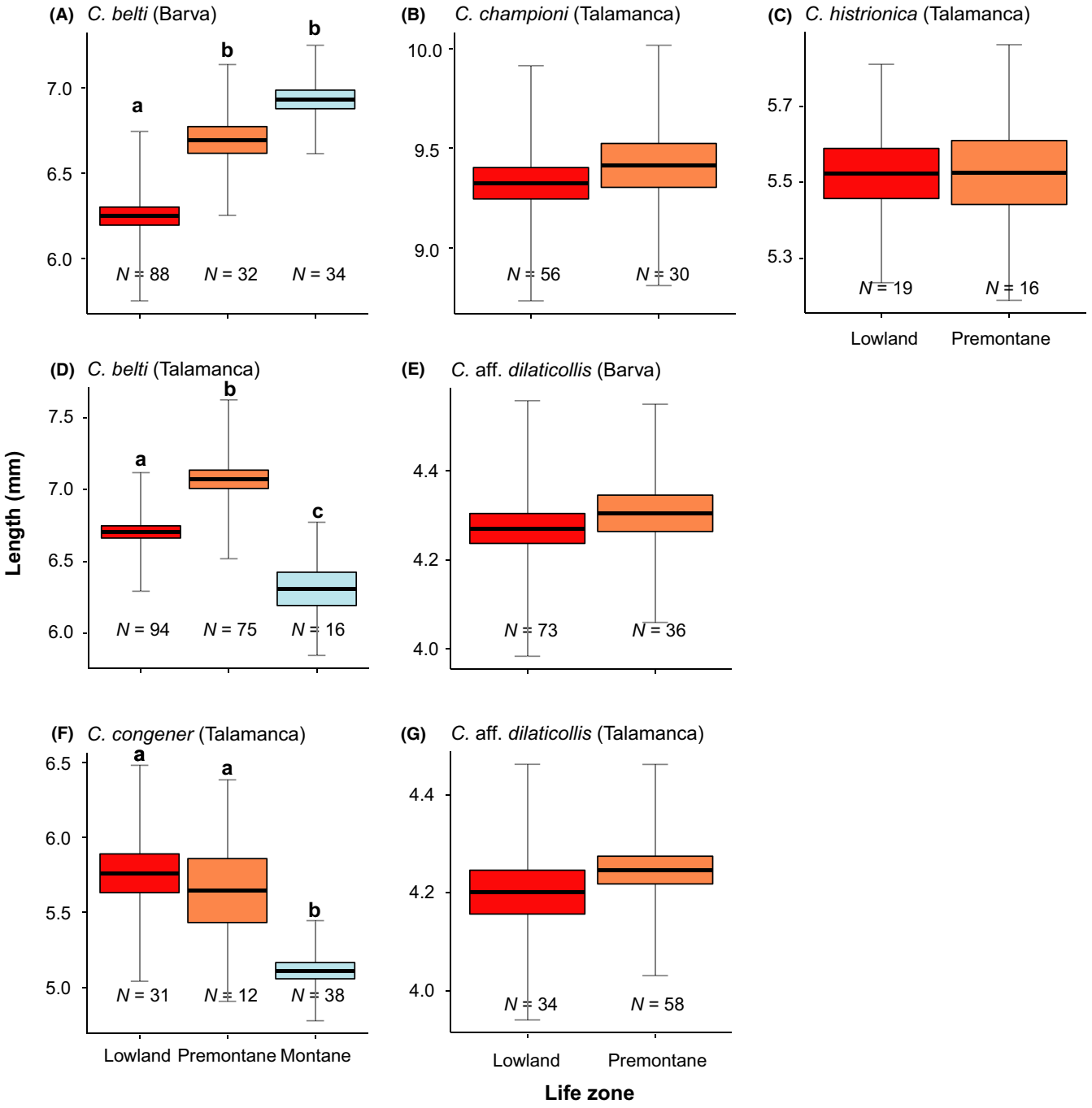

FIGURE 3 Lengths ($M \pm SE \pm SD$) of *Cephaloleia* species occurring in multiple life zones along elevational gradients. Letters indicate significantly different groups ($p < 0.05$). (A) *C. belti* on Barva. (B) *C. championi* on Talamanca. (C) *C. histrionica* on Talamanca. (D) *C. belti* on Talamanca. (E) *C.* aff. *dilaticollis* on Barva. (F) *C. congener* on Talamanca. (G) *C.* aff. *dilaticollis* on Talamanca. Total number of individuals = 798, see sample size in Supporting Information S2

Body size is influenced by many factors, so perhaps it is not surprising that we found body size differences between *Cephaloleia* species were better predicted by shared evolutionary history than by temperatures associated with their life zone or other factors. While all the species we considered are congeners with the same habit of living in unfurling young leaves, the largest beetle species measured was more than three times as long as the smallest (*C. stevensi*: 3.5 mm, *C. heliconiae*: 11.1 mm). One life-history factor that is shared among closely related species and may contribute to this variation is host plant, specifically host plant leaf size. These larvae feed in the expanding leaves, predominately belonging to plants in the families Costacaeae, Heliconiaceae, Marantaceae and Zingiberaceae. While Costa Rican Heliconiaceae and many Marantaceae have large leaves (0.5–3 m long), most Costaceae have mature leaves 0.1–0.5 m long (Hammel, Grayum, Herrera, & Zamora, 2010). Small leaves not only offer less leaf tissue but also expand more rapidly than large leaves. *Cephaloleia* larvae cannot consume mature leaf tissue and must pause their development until a new leaf emerges (Strong, 1982).

All else being equal, an individual would take longer to reach the same size on a plant with smaller leaves. This limitation likely explains why small *Cephaloleia* species are usually found on host plants with small young leaves. For example, the smallest beetle species in our study *C. stevensi* is found on the Marantaceae with the smallest size, *Goeppertia micans*. As both large, and small-leaved host plants are found at all elevations in this study, this relationship between leaf size and beetle size may explain why all beetle assemblages had similar mean body sizes.

Our hypothesis that there would be intraspecific variation in size with elevation was better supported, probably because intraspecific comparisons control for evolutionary history. However, size

TABLE 3 ANOVAs and *t* tests for beetles reared at different temperatures

Species	Test statistic	*df*	*p*
Cephaloleia belti (lowland)	$t = -1.014$	199.96	0.31
Cephaloleia belti (montane)	$t = -0.475$	31.927	0.64
Cephaloleia aff. *dilaticollis* (lowland)	$t = 3.723$	145.24	$\mathbf{2.8 \times 10^{-4}}$
Cephaloleia aff. *dorsalis* (lowland)	$t = 0.151$	251.39	0.88
Cephaloleia placida (lowland)	$F = 9.774$	2, 197	$\mathbf{2.2 \times 10^{-4}}$

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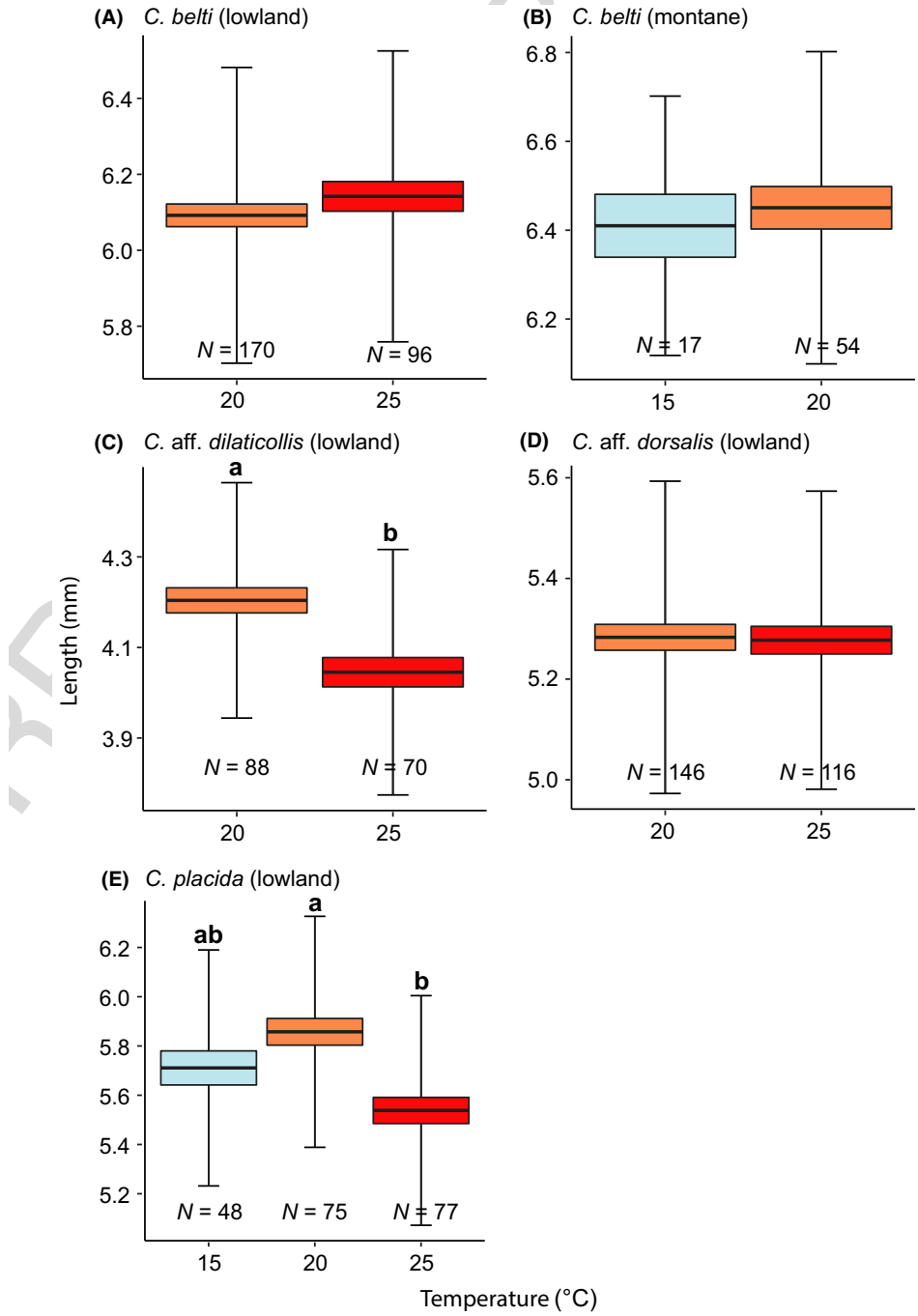

FIGURE 4 Lengths ($M \pm SE \pm SD$) of beetles reared at different temperatures. Temperatures are comparable to the mean temperatures of life zones in Figures 1–3, as indicated by bar colour. Letters indicate significantly different groups ($p < 0.05$). (A) Lowland *C. belti*. (B) Montane *C. belti*. (C) Lowland *C.* aff. *dilaticollis*. (D) Lowland *C.* aff. *dorsalis*. (E) Lowland *C. placida*. Host plants used to feed each beetle species: *Cephaloleia belti: Heliconia latispatha* (Heliconiaceae), *C. dilaticollis* and *C. placida: Renealmia alpinia* (Zingiberaceae), *C. dorsalis: Costus malortieanus* (Costaceae). Total number of individuals = 968, see sample size in Supporting Information S3

differences with elevation were only seen in species that occur in all three life zones and were not always consistent with the prediction of small size at warmer temperatures. There was only one instance (*C. belti* on Barva) where beetles uniformly increased in size with increasing elevation. In the other cases, beetles were smaller at montane elevations, but had different size patterns at intermediate elevations. Species represented by only lowland and premontane populations did not differ in size. The intraspecific similarity of species that only occur below 1,500 m suggests that rather than a continuous relationship between elevation and body size, there is a distinct threshold above which a *Cephaloleia* species experiences different pressures on body size.

These pressures on body size apparently vary among and even within species. If we accept the axiom that larger conspecifics have greater fecundity, then montane populations with smaller individuals (*C. belti* and *C. congener* on Talamanca) may indicate that the beetles cannot grow to full size at high elevations due to cold stress or other factors. The *C. belti* populations found on the Barva gradient, however, have large montane individuals. Our laboratory experiments showed that Barva *C. belti* montane and lowland populations are thermal specialists and cannot successfully develop at each other's mean temperatures. Furthermore, the size differences between the two populations persist even when reared at different temperatures, indicating that these differences are heritable rather than the result of body size plasticity. Previous results show that such responses can be attributed to both plasticity and fixed traits (Berner & Blanckenhorn, 2006; Chown & Klok, 2003; Dittrich, Drakulić, Schellenberg, Thein, & Rödel, 2016; Walczyńska & Serra, 2014). How the Talamanca populations of *C. belti* and *C. congener* perform at different temperatures is unknown. Further investigation of their thermal specialization, and in the case of *C. belti*, the evolutionary relationships among the different populations are necessary to understand why their size–elevation relationships differ.

Our hypothesis that beetle populations would exhibit body size plasticity when reared at different temperatures was supported for two life zone specialist species only. *C.* aff. *dilaticollis* and *C. placida* displayed in some cases the expected 'hotter is smaller' pattern. However, lowland *C.* aff. *dorsalis* reached the same adult size regardless of temperature, as did the two *C. belti* populations. Since larvae of all populations developed more rapidly at warmer temperatures (unpubl. data), the *C. belti* and *C.* aff. *dorsalis* individuals must have grown faster to reach the same adult size. Taken together, this suggests that some *Cephaloleia*, especially life zone generalists, use growth rate plasticity to reach a fixed adult body size regardless of temperature. Life zone specialists have little pressure to regulate their growth rate over different temperatures and may therefore display size plasticity.

Data on intraspecific size change with latitude or elevation are rare for tropical insects (but see Brehm, Zeuss, & Colwell, 2019; Hawkins & DeVries, 1996). More generally, a recent meta-analysis of intraspecific insect size variation across elevation found that elevation–size relationships were much more heterogeneous than either latitudinal- or rearing temperature–size relationships (Horne, Hirst, & Atkinson, 2018). Suggested explanations for this heterogeneity include the effects of oxygen availability, voltinism and population mixing (Dillon et al., 2006; Verberk & Bilton, 2011; Verberk, Bilton, Calosi, & Spicer, 2011; Verberk et al., 2020). Our study cannot directly address the effects of oxygen stress, as only the montane *C. belti* reared in the lowlands were exposed to novel oxygen levels. This question would be best addressed using experiments that independently vary temperature and oxygen partial pressure for multiple populations.

However, we can offer some insights into the potential role of voltinism (i.e. the number of generations per year; Gullan & Cranston, 2014). *Cephaloleia* beetles are multivoltine, with individuals living for over 2 years, but they also experience an unrestricted growing season due to their relatively aseasonal habitats. As a result, generations overlap and partial generations continue their development in the next calendar year rather than being cut short at the end of the growing season. In areas with finite growing seasons, however, relationships between season length, development time and fitness can be complex (Roff, 1980). Univoltine insects in seasonal environments may either extend their growth over the entire growing season or only be active for a portion of it (e.g. eastern tent caterpillars only feed on expanding spring leaves and spend most of each year inactive; Fitzgerald, 1995). In such cases, adult body size will likely depend on conditions during the active growth period rather than the rest of the year. Similarly, multivoltine insects in seasonal environments can have development times ranging from weeks to months. Taxa with longer development times will likely experience trade-offs unless they are able to develop over multiple growing seasons (Blanckenhorn & Demont, 2004; Zeuss, Brunzel, & Brandl, 2017).

Our results suggest that beetles with unlimited growing seasons can frequently maintain a constant body size over different temperatures or elevations by varying their growth and development. Similarly, flies, which have very short development times, do not display significant elevation–size relationships (Horne et al., 2018). This suggests that elevation–size relationships could be insignificant in insects whose development time is short relative to the length of the growing season. However, a study of weevils found that high-elevation individuals were larger than lower-elevation conspecifics on a relatively aseasonal temperate elevational gradient (Chown & Klok, 2003). More body size data need to be put into the context of seasonality to test whether long growing seasons lead to nonsignificant elevation–size relationships because of the plasticity they allow. If this hypothesis is correct, we would expect elevation–size correlations to occur on temperate or otherwise seasonal mountains in slow-developing taxa, but not on aseasonal tropical mountains, regardless of development rate.

The variation in the intraspecific elevation–size relationships seems unlikely to be due to differences in population mixing between life zones. A tropical mountain compresses the temperature variation of a tropics-to-pole latitudinal gradient into tens of

kilometres (Rahbek et al., 2019). These short distances raise the possibility that individuals could move between life zones, and some tropical insects are even known to be elevational migrants (Haber & Stevenson, 2004). However, non-migratory taxa may be too locally adapted to survive large elevational movements (Janzen, 1967), as exemplified by the inability of the two *C. belti* populations to survive at each other's local temperatures in the laboratory. Such long-distance travel is also unlikely. *Cephaloleia* beetles are not particularly strong fliers and, as understory beetles feeding inside rolled leaves, are unlikely to be exposed to strong winds. While this does not rule out short-distance dispersal and gradual gene flow between life zones, there would need to be very distinct dispersal patterns or gene flow effects to cause the different elevation–size relationships displayed by the Barva *C. belti*, the Talamanca *C. belti* and the *C. congener* populations. At the same time, there would need to be very uniform gene flow between all the lowland-premontane populations that do not show size differences between life zones. While gene flow mechanisms could be tested using population genetics techniques, they seem unlikely to account for all the observed patterns.

An alternative hypothesis that has not been tested is whether these intraspecific elevation–size patterns can be explained by variation in the nutritional quality of host plants present at each elevation. In previous studies, we reared *Cephaloleia* beetles on host plants with contrasting nutritional qualities and found that body size was always smaller on low-quality hosts (García-Robledo & Horvitz, 2011, 2012). These results suggest that diet composition has a strong effect on insect body size, potentially masking any effects of temperature on this particular trait.

5 | CONCLUSIONS

Our investigations of the relationships between body size, temperature and elevation in *Cephaloleia* beetles highlight the importance of comparing these relationships across multiple scales. At the interspecific level, our phylogenetic analysis demonstrates that body size is predicted by evolutionary history rather than elevational life zone. This reinforces previous suggestions that temperature–size relationships are best analysed through intraspecific comparisons (Shelomi, 2012) and suggests that, as more genetic resources become available, past interspecific comparisons should be reanalysed using phylogenetic methods. If previously reported interspecific temperature–size patterns are generally artefacts of the assumption that species are evolutionarily independent, then biologists should focus on investigating intraspecific temperature–size relationships. At the intraspecific level, we demonstrated that different species manifest different elevation–size relationships and that these are sometimes the result of heritable differences rather than body size plasticity. This provides further evidence against a general thermodynamic or metabolic explanation for ectotherms with negative temperature–size relationships. At an applied level, this offers some good news: we should not assume that global warming will inevitably lead to body size decreases for ectotherms. However, the absence of a general temperature–size relationship means that if we do want to predict how ectotherm body size will respond to global warming, a great deal of specific research will be required.

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AUTHORS' CONTRIBUTIONS

C.G.-R. secured funding, conceived the project, collected and identified beetles, sequenced DNA, curated the data bases, coordinated laboratory work, designed figures, conceived and corrected analyses and wrote the final version of the manuscript; C.S.B. performed the interspecific phylogenetic analyses and the laboratory rearing analyses, prepared a first draft of the figures and prepared a preliminary draft of the manuscript; K.L. and V.S. measured the wild-caught beetles, performed the intraspecific size analyses and wrote a first version of that portion of the manuscript. All authors gave final approval for the publication.

DATA AVAILABILITY STATEMENT

The phylogenetic data are deposited in TreeBASE and the size data have been included as part of the supplementary material. DNA sequences deposited in GenBank, database, https://doi.org/10.5883/DS-BOFCR, accession nos. KU357054–KU358485.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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