Elevational clines in morphological traits of subtropical and tropical butterfly assemblages

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Morphological traits can determine the ecological niches and performance of ectotherms and structure their distributions along environmental gradients. The thermal melanism hypothesis and Bergmann's rule describe patterns of body colour luminance and body size along environmental gradients shaped by thermal influences on morphology. However, these patterns have rarely been investigated at the interspecific level for subtropical and tropical mountain environments. In this study, we sampled butterfly assemblages along elevations across three subtropical and tropical locations in China and examined how environmental factors affected body colour luminance and body size. We additionally reconstructed phylogenetic relationships among the sampled butterfly species and investigated morphology–elevation relationships within an evolutionary framework. Butterfly assemblages were consistently darker and larger at higher elevations across three replicate locations. Furthermore, based on a phylogenetic comparative analysis, we found that body colour luminance and body size of butterfly assemblages responded to elevation through both long-term processes and more recent environmental influences. Our findings support the thermal melanism hypothesis and Bergmann's rule from diverse subtropical and tropical butterfly assemblages, indicating elevation may structure the distributions of tropical species through morphology. The thermal functions of morphology should therefore be considered when investigating species distribution patterns and responses to environmental changes.

ADDITIONAL KEYWORDS: butterfly – body size – diversity – elevation – solar radiation – subtropical – temperature – thermal adaptation – thermal melanism – tropical.

INTRODUCTION

The vulnerability of species to global warming depends on their intrinsic characteristics (sensitivity) and the magnitude of environmental changes across their distributions (exposure) (Williams *et al.*, 2008). Ecological traits including morphological, physiological and phenological features have the potential to buffer climate change threats to species and can therefore contribute to prediction of species and community responses to environmental changes (Lavergne *et al.*,

2010; Buckley & Kingsolver, 2012; Diamond *et al.*, 2012; Garcia *et al.*, 2014; Ficetola *et al.*, 2016). Regional environmental gradients, such as elevation, can create wide environmental variation, which can structure trait variation in species or communities (McGill *et al.*, 2006; Peters *et al.*, 2016). Therefore, investigating trait variation along environmental gradients can reveal mechanisms of community assembly and patterns of ecological processes, such as environmental filtering and species adaptation, critical for understanding biodiversity responses to environmental changes (McGill *et al.*, 2006; Cavender-Bares *et al.*, 2009).

Spatial patterns of thermally important morphological traits have been found within and across

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species along environmental gradients (Zeuss *et al.*, 2014; Moreno Azócar *et al.*, 2015; Peters *et al.*, 2016). Two notable hypotheses illustrate the importance of morphological patterns across environmental gradients: the thermal melanism hypothesis (hereafter TMH) suggests that darker organisms are more efficient in transferring solar energy to body heat gain, a benefit in cool climates (Clusella-Trullas, van Wyk & Spotila, 2007; Clusella-Trullas *et al.*, 2008); and Bergmann's rule proposes that large body size contributes to heat maintenance, resulting in a general increase in body size or mass in colder climates (Bergmann, 1848; Partridge & French, 1996). While the thermally adaptive basis of morphology remains of debate, with contrasting evidence across taxa and geographical gradients (Ashton & Feldman, 2003; Shelomi, 2012; Peters *et al.*, 2016; De Souza *et al.*, 2017; Stuart-Fox, Newton & Clusella-Trullas, 2017), recent climate warming has been documented as a force in driving distribution shifts through the filtering of species based on body colour and size (Gardner *et al.*, 2011; Sheridan & Bickford, 2011; Zeuss *et al.*, 2014). Therefore, understanding how the TMH and Bergmann's rule may apply to regional species assemblages along environmental gradients could provide key insights into assessments of climate change vulnerability. As related species tend to share similar ancestral traits and occur in similar environments (and thus may respond similarly to thermal gradients), phylogenetic relatedness must also be accounted for in trait analyses (Cavender-Bares *et al.*, 2009; Wiens *et al.*, 2010; Buckley & Kingsolver, 2012).

The TMH and Bergmann's rule have been largely tested either within species at local scales or based on species occurrence at large geographical scales (Kingsolver, 1983; Zeuss *et al.*, 2014; Pinkert, Brandl & Zeuss, 2017). Little evidence has been shown from field observations at assemblage levels along continuous elevation profiles, especially in the tropics where high levels of biodiversity may be particularly vulnerable to warming (Deutsch *et al.*, 2008; Bonebrake & Deutsch, 2012; Xing *et al.*, 2016). Butterflies represent an ideal system in testing the TMH and Bergmann's rule for tropical ecototherms (Watt, 1968; Wasserthal, 1975; Kingsolver, 1985, Bonebrake *et al.*, 2010). Previous physiological experiments have demonstrated that colour luminance and body size are important traits in butterfly thermoregulation, and dark colours and large body sizes will significantly raise body temperature when the animal is exposed to the sun in stable environmental conditions (Watt, 1968; Wasserthal, 1975; Kingsolver, 1985; MacLean *et al.*, 2016; Xing *et al.*, 2016). Intraspecific thermal melanism has also been observed in both common garden experiments and along thermal gradients in several butterfly

species (Ellers & Boggs, 2002; Davis, Farrey & Altizer, 2005; Karl, Geister & Fischer, 2009).

In this study, we aim to test the TMH and Bergmann's rule on butterflies at the interspecific level along elevational gradients in the tropics. We hypothesized that there would be morphological patterns across elevation gradients for tropical butterflies, and that darker and larger butterflies should be favoured by cool (high elevations) over hot (low elevations) environments. Moreover, we hoped to gain insights into how evolution has shaped these patterns. We investigated forest butterfly assemblages and the environments they experience along elevational gradients in three locations of subtropical and tropical China. Based on field community sampling and specimen measurement, we conducted a species-specific morphological analysis, and applied a phylogenetic framework to account for phylogenetic effects in trait variation. The results offer insights into how thermally important morphological traits may structure species distributions and affect climate change responses of highly diversified subtropical and tropical ectotherm assemblages.

MATERIAL AND METHODS

BUTTERFLY SAMPLING AND ENVIRONMENTAL DATA COLLECTION

We sampled butterfly assemblages in three locations in subtropical and tropical China including Mao'er Mountain National Nature Reserve in Guangxi (25°51′59.42″N, 110°24′46.05″E: subtropical evergreen broadleaf forests), five protected areas in Hong Kong (22°15′0″N, 114°10′0″E: subtropical evergreen broadleaf forests) and Xishuangbanna National Nature Reserve in Yunnan (21°36′42″–58″N, 101°34′26″–47″E: tropical rainforests) (Supporting Information, Fig. S1) during the wet season when most butterflies are active. The three locations were chosen not only because of their high levels of biodiversity in Asia, but also because they are covered with well-preserved forests across large spans of elevational range (Bascombe, Johnston & Bascombe, 1999; Chen, 2001; Wang & Tang, 2012). In Mao'er Mountain National Nature Reserve, field sampling was conducted from 14 to 27 September 2015, covering elevations from 500 to 2000 m. In Hong Kong, field sampling was conducted in five areas (Victoria Peak, Tai Po Kau Nature Reserve, Shingmun Country Park, Pok Fu Lam Reservoir Country Park and Tai Mo Shan Country Park) from 10 June to 8 July 2015, covering elevations from 170 to 673 m. In Xishuangbanna National Nature Reserve, field sampling was conducted in three areas (Xishuangbanna Botanical Garden, Bubeng

and Nangongshan station) during 17–31 July 2015 and 22–28 August 2015, covering elevations from 550 to 1500 m. In total, we surveyed 38 days and 88.5 h including 32.5 h within 11 sampling days in Guangxi, 19.5 h within 10 sampling days in Hong Kong and 36.5 h within 17 sampling days in Yunnan (see Table S1 for detailed transect information).

Within each location, we collected specimens in late morning and early afternoon on sunny and low wind days by walking 0.5-km-long transects, each within 30 min following standardized Pollard walk methods (Pollard, 1977). We identified species in the field by observation using visual inspection and by catching specimens using hand-nets. We recorded the abundance for each species during sampling. For each individual, we recorded the time it was encountered to track the instant air temperature and solar radiation from our data logger of the transect afterwards (see below). We also noted whether the individual occurred under sunlight by categorizing the spot where the individual was sighted into sun or shade conditions. All specimens collected were mounted for further morphological measurement. Overall, we sampled 184 species from 2500 individuals, including 1227 individuals of 80 species in Guangxi, 554 individuals of 55 species in HK, and 719 individuals of 101 species in Yunnan.

To measure the air temperatures and solar radiation butterflies experienced in the forests at each transect, at least one data logger (HOBO UA-002-64, Onset Computer Corporation, Bourne, MA, USA) was placed 1 m above the ground within each transect. The data loggers were programmed to measure ambient air temperature and light intensity at 5-min intervals during the sampling period. Measured light intensity values were converted to solar radiation for further statistical analysis according to the relationship summarized by Johnson & Wilby (2013). We calculated hourly mean air temperatures and solar radiation recorded at each transect and used a multiple linear regression to quantify how hourly mean air temperatures and solar radiation were related to elevation and location. We found that both air temperature and solar radiation varied across locations, but showed a general elevational pattern, with a significant decrease in air temperature ($F_{3,2456} = 2064$, $P < 0.001$) and increase in solar radiation ($F_{3,1226} = 78.32$, $P < 0.001$) with increasing elevation (Fig. S2).

MORPHOLOGICAL ANALYSIS

We used specimens from existing collections housed at Xishuangbanna Botanical Tropical Garden (collected in 2014–2015) and South China Agriculture University (collected in 2003–2012), as well as our own collections during field sampling, to acquire the colour luminance and body size information for each species.

To measure colour luminance using a standardized and quantitative approach, we photographed each specimen with the same camera (Nikon D5300) under dark conditions using a standardized setting (exposure time: 1/60 s, ISO speed: 125, aperture: F/16) from a fixed distance (45 cm). We took photos of both the dorsal and the ventral sides of the wing for each sample. Previous research has indicated that the region most related to thermoregulation for butterflies is typically the body together with the basal part of the wings (Wasserthal, 1975). We therefore chose the body plus basal one-third of the wing area to analyse colour luminance (similar to Zeuss *et al.*, 2014; Xing *et al.*, 2016). We used Adobe Photoshop CC 2014 software to transfer each image to greyscale using the *blur* function and then averaged the luminosity [a value between 0 (dark) and 255 (light)] of all pixels covering the chosen region. All specimens were mounted in a standardized manner, with the hind margin of the forewing forming a straight line perpendicular to the body. We used digital calipers (0–100 mm) to measure wingspan (the distance between the tips of the forewings) as a proxy of body size (Sekar, 2012). Although intraspecific variation in traits can be important for some butterfly species (e.g. Ellers & Boggs, 2004), in this study we focused on the morphology across species (interspecific variation) as this is probably the more important component of trait variation at the assemblage level (McGill *et al.*, 2006; Gaston, Chown & Evans, 2008). We averaged morphological values for each species based on collected and existing specimens when multiple specimens were available. Due to limitations in the identification of sex through field observation, we did not record the sex of each individual encountered. We averaged the morphological information across sex when we had specimens for both sexes. Thus, in our analysis, all individuals belonging to one species share the same morphological information. This might cause some bias in our results if sex ratios differ significantly from 1:1 or there are consistent differences across sexes (e.g. female butterflies are typically larger than males; Chou, 1994). However, overall assemblage patterns are probably robust to these effects due to the relatively small magnitude of between-sex variation compared to across-species variation (Zeuss, Brunzel & Brandl, 2017). We obtained dorsal colour luminance for 166 species, ventral colour luminance for 165 species and wingspan for 168 species (Table S2).

PHYLOGENETIC RECONSTRUCTION AND ANALYSIS

Among all the species found in the sampling locations, we obtained sequences from ten genes regions for 139 (out of 184 species recorded in this study) from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). We used these

sequences for phylogenetic reconstruction (see Fig. S3 for details). We extracted 300 trees from post burn-in trees in one of the Monte Carlo Markoc chain (MCMC) runs. For traits sampled by each tree, we used Pagel's lambda (Pagel, 1999) to calculate the phylogenetic signal with the *phylosig* function in the R package *phytools* (Revell, 2012). Pagel's lambda assumes the evolution of a trait follows the Brownian motion model, and it lies between zero and one. A Pagel's lambda closer to zero shows low phylogenetic signal, indicating phylogenetic independence, while a value closer to one shows strong phylogenetic signal, indicating species traits are distributed under Brownian motion (Pagel, 1999).We then rescaled the trait values with Pagel's lambda using the *rescale* function in the R package *geiger* (Harmon *et al.*, 2008). We then applied Lynch's comparative method (Lynch, 1991) to separate the trait values into phylogenetic components (ancestrally influenced part of the trait) and specific components (species-specific deviation part of the trait), based on 300 trees extracted with the function *compar.lynch* in the R package *ape* (Paradis, Claude & Strimmer, 2004). In this way, we calculated 300 phylogenetic components and specific components for each species.

STATISTICAL ANALYSIS

We matched morphological data to environmental information including elevation, location, microclimate (sun/shade) and weather (air temperature, solar radiation) for each individual and pooled data collected from the three locations together. We then ran multiple linear models to test the effects of different environmental factors including weather (solar radiation and ambient temperature) and elevation as continuous factors, and location and sun/shade condition as categorical factors on the morphology of butterfly individuals. We then applied an Akaike information criterion (AIC) for the model selection process to select the best-fit models (lowest AIC value) to choose the most important factors in shaping colour luminance and size of butterflies (Akaike, 1974) using the R package *MuMIn* (Bartoń, 2013). In addition, we treated the occurrence of a species as a replicate and conducted a multiple linear regression to investigate how colour luminance and body size of the species presented were affected by elevation and location.

To control for phylogeny effects on traits, we also applied the same best model we used when investigating the morphology and environment relationship at the individual level to explore how environmental factors shaped the phylogenetic and specific components of the colour luminance and body size of butterfly individuals across elevation. We ran the models based on a subset of the dataset due to limitations in the phylogenetic information for some species ($N = 139$).

We treated each individual as a replicate, but instead of using the actual morphological information as a response variable, we used phylogenetic components and specific components. We ran the same analysis for all 300 pairs of phylogenetic components and specific components, and averaged results of 300 models as the final model result. All analyses were conducted in R v.3.2.5 (R Core Team, 2013).

RESULTS

Results from model selection suggest that among all the environmental factors, air temperature, solar radiation, elevation and location affected colour luminance of butterfly individuals, while sun/shade condition in addition to those factors affected wingspan (body size) of butterfly individuals (Tables S3 and S4). Both colour luminance and wingspan varied across the three locations, with butterflies in Hong Kong being the darkest and largest (Table 1). The relationship between traits and elevation, however, were consistent among different locations (Figs 1, 2). Overall, using individuals as response units, elevation had significant negative effects on colour luminance of both sides for butterfly individuals, suggesting that individuals were darker with increasing elevation (Table 1 and Fig. 1). In addition, solar radiation positively influenced colour luminance on both dorsal and ventral sides of butterfly individuals. Air temperature also showed positive effects on both sides of butterflies but the effect was only significant for the dorsal side (Table 1). As solar radiation and air temperature represented the microclimate of each transect as well as the temporary weather conditions when each individual was sampled, their effects together indicate that lighter individuals prefer warm and sunny conditions (Table 1). Elevation showed significant positive effects on the wingspan of individuals, suggesting individuals were larger at higher elevation (Table 1 and Fig. 2). While sunny conditions tended to favour large individuals in general, the prevalence of small individuals increased towards stronger solar radiation (Table 1). No significant effects of air temperature were observed on wingspan (Table 1). When using the occurrence of one species as a replicate, we found no significant effect of elevation on luminance of the dorsal wing, but a nearly significant negative effect of elevation on luminance of the ventral wing. We found a significant positive effect of elevation on wingspan. This indicates species with darker ventral wings and larger sizes tend to occur at higher elevations (Table 1).

The phylogenetic relationship of butterfly species with phylogenetic information ($N = 139$) is summarized in Fig. S3. Strong phylogenetic signals were observed in both colour luminance and wingspan

Table 1. Results of best multiple linear regression models for relationships between traits and environmental factors at individual and species levels

Traits	d.f.	Parameter	Estimate	SE	*t* value	Pr(>|*t*|)
Unit: individual						
Dorsal colour luminance	2272	Solar radiation	0.062	0.023	2.670	0.008**
		Air temperature	0.625	0.318	1.967	0.049*
		Elevation	−0.008	0.003	−3.152	0.002 **
		Hong Kong	−45.378	2.369	−19.154	<0.001**
		Yunnan	−19.532	1.679	−11.631	<0.001**
Ventral colour luminance	2266	Solar radiation	0.069	0.023	3.000	0.003 **
		Air temperature	0.429	0.320	1.340	0.180
		Elevation	−0.019	0.003	−7.162	<0.001**
		Hong Kong	−59.622	2.380	−25.051	<0.001**
		Yunnan	−25.971	1.687	−15.393	<0.001**
Wingspan	2261	Solar radiation	−0.042	0.015	−2.794	0.005 **
		Air temperature	−0.177	0.209	−0.848	0.396
		Elevation	0.018	0.002	9.910	<0.001**
		Sun	2.710	1.042	2.599	0.009 **
		Hong Kong	19.120	1.533	12.476	<0.001**
		Yunnan	−7.894	1.073	−7.355	<0.001**
Unit: species						
Dorsal colour luminance	821	Elevation	−0.003	0.004	−0.883	0.378
		Hong Kong	−30.628	4.147	−7.384	<0.001**
		Yunnan	−12.277	3.131	−3.920	<0.001**
Ventral colour luminance	811	Elevation	−0.007	0.004	−1.804	0.0716
		Hong Kong	−37.855	4.112	−9.204	<0.001**
		Yunnan	−17.812	3.095	−5.754	<0.001**
Wingspan	805	Elevation	0.014	0.002	5.968	<0.001**
		Hong Kong	20.060	2.560	7.836	<0.001**
		Yunnan	3.076	1.947	1.580	0.115

*$P < 0.05$; **$P < 0.01$.

of butterfly species with lambda values all higher than 0.8 (closer to 1) (Fig. 3). In addition, for individual colour luminance, we found the same pattern for both phylogenetic and specific components of dorsal and ventral colour luminance, showing a decreased trend (darker) with increased elevation (Table S5 and Fig. 4). We found the same pattern for both phylogenetic and specific components of wingspan, which increased with elevation (Table S5 and Fig. 5).

DISCUSSION

We found a consistent pattern across three locations in subtropical and tropical China that butterflies were darker and larger at higher elevations. This observed climatic cline in butterfly morphology provides evidence supporting the TMH and Bergmann's rule from subtropical and tropical mountain regions. The environment–morphology relationship found in this study is similar to that found in other recent studies on ectotherms across large latitudinal gradients (Zeuss *et al.*, 2014; Moreno Azócar *et al.*, 2015; Peters *et al.*, 2016) and to previous findings at the habitat level in the tropics (Xing *et al.*, 2016), which highlights the role of abiotic factors in influencing the abundance and distribution of insects from subtropical and tropical mountains. The sensitivity of morphological traits to climatic factors indicates potential vulnerability of tropical butterflies to climate change.

Solar radiation and ambient temperature could have direct and substantial influences on thermoregulation and activity of ectotherms through behaviour, morphology and the interplay between them (Ohsaki, 1986; Bonebrake *et al.*, 2014; Stuart-Fox, Newton & Clusella-Trullas, 2017). We found that butterflies tended to be lighter-coloured and smaller under strong solar radiation. This could be explained by a limited ability to thermoregulate for lighter and smaller butterflies, which are more dependent on high solar radiation levels for flight due to their slower warm-up rate and higher rate of convective heat loss (Church, 1960; Gilchrist, 1990; Pereboom & Biesmeijer, 2003; Xing *et al.*, 2016).
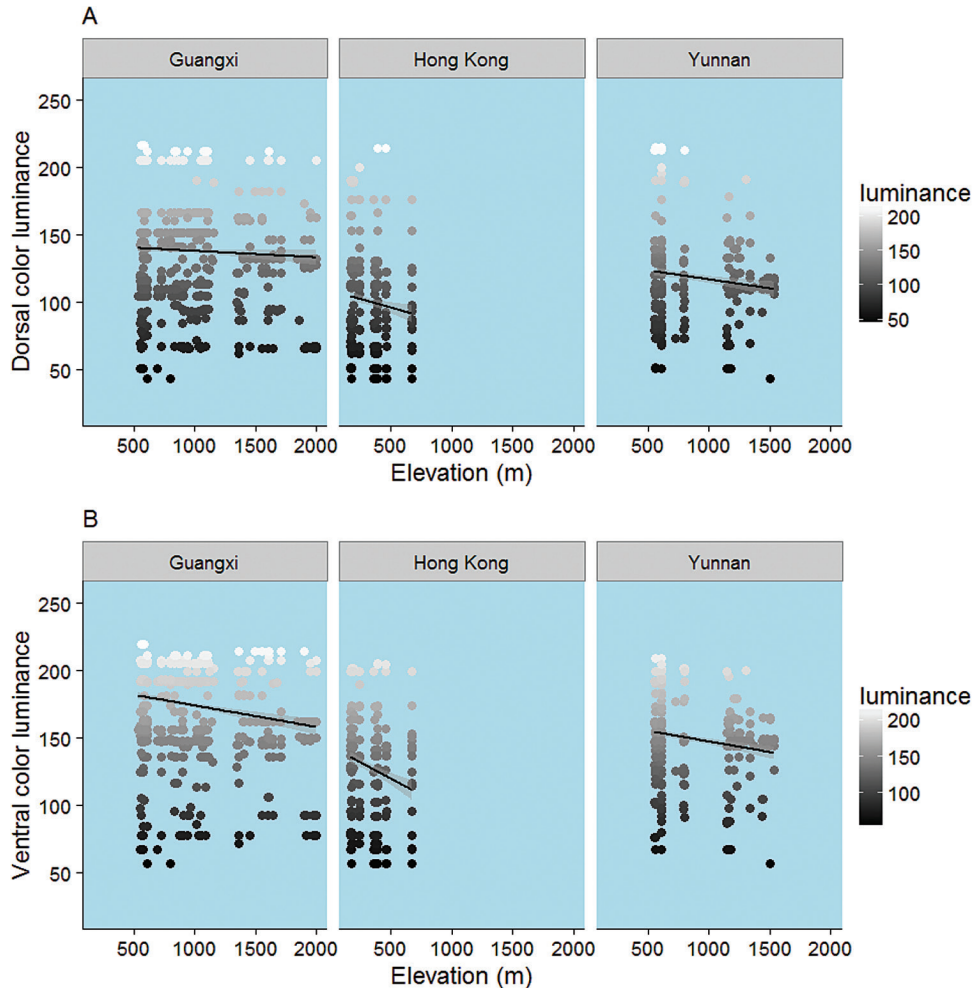
Figure 1. Colour luminance of dorsal (A) and ventral sides (B) of butterflies along elevations across three locations. Each dot represents one individual; darker dots indicate darker species. Solid lines show the mean relationship with standard errors (grey shaded areas) between colour luminance of butterflies and elevation within each location.

Besides environmental effects, we observed deep evolutionary influences on morphological traits of subtropical and tropical butterfly species. The high lambda value (close to 1) of colour luminance and wingspan showed a strong phylogenetic signal in both of the traits, indicating a tendency for species to retain their ancestor's morphological characters over time (Harvey & Pagel, 1991; Pagel, 2002). No strong correlation was found between body colour luminance and size (Fig. S4), which suggests that colour luminance and body size may develop independently through thermal adaptation along elevation gradients. The high phylogenetic signal on colour luminance of Asian butterflies is similar to that found for colour luminance of butterflies in Europe (Zeuss et al., 2014), suggesting a conservatism in butterfly colour luminance through evolutionary processes. Similarly, a strong phylogenetic signal (Pagel's lambda) of colour luminance was also detected among dragonfly species

(Pinkert, Brandl & Zeuss, 2017), but not among ant species (Bishop et al., 2016). Therefore, the influence of evolutionary history on colour luminance may vary across different taxa and should be considered when examining environment–colour luminance relationships.

As both phylogenetic and specific components of colour luminance and body size responded to elevation, the trait distribution of the butterfly assemblages may result from a mixed effect of conservatism and convergence (Webb et al., 2002). In this case, environmental niches (e.g. cool climates at high elevations) created by environmental factors (elevation in this case) may have been filled as a result of both *in situ* evolution of species from a certain lineage with morphological traits that fit in the niche and colonization by species of different lineages that share similar traits (Webb et al., 2002; Cavender-Bares et al., 2009). These phylogenetic influences on
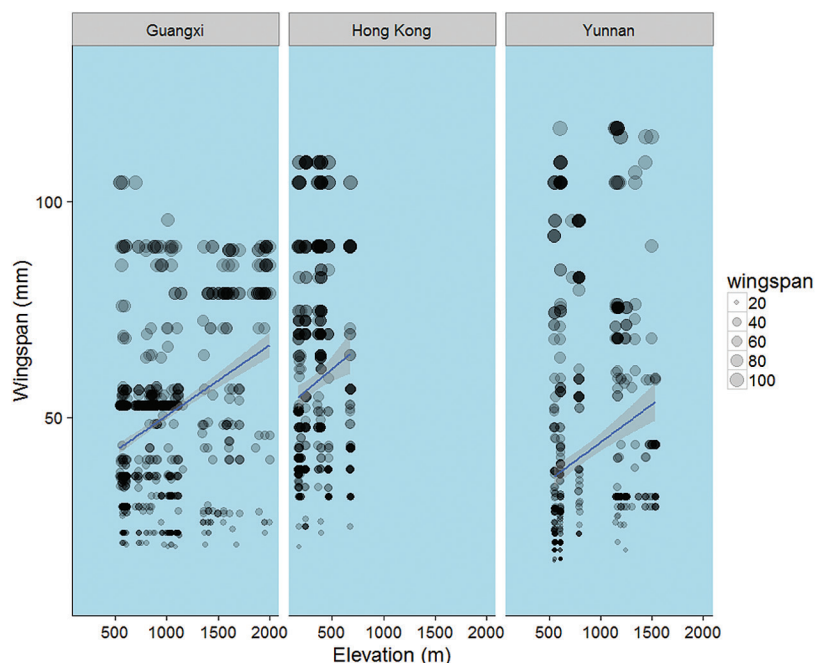
Figure 2. Wingspan of butterflies along elevations across three locations. Each dot represents one individual; larger dots indicate larger species, and darker dots indicate relatively higher frequency of the species. Solid lines show the mean relationship with standard error (grey shaded area) between butterfly wingspan and elevation within each location.
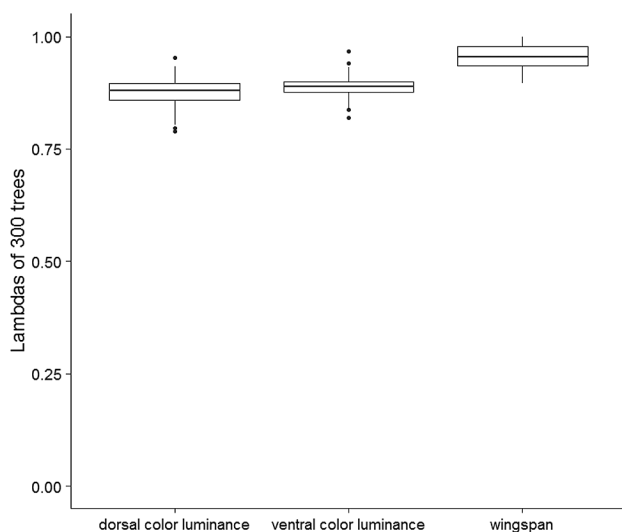

Figure 3. Phylogenetic signal (lambda) calculated based on 300 phylogenetic trees for butterfly dorsal colour luminance, ventral colour luminance and wingspan. A lambda value closer to one indicates high phylogenetic signal among species traits. Boxplots show medians with overall variances of lambda values for each trait.

thermally adaptive traits could render tropical species vulnerable to warming and potentially hinder fast evolving trait adaptation that might otherwise protect these species.

Although between-species trait variance could be more useful when investigating traits at assemblage levels (McGill *et al.*, 2006), intraspecific trait variation may provide additional information on trait–environment relationships and improve our understanding of ecological and evolutionary processes (Violle *et al.*, 2012; Classen *et al.*, 2017). Plasticity has been observed in butterfly colour luminance and size as a response to climate (Ellers & Boggs, 2002; Juhász *et al.*, 2016; Klockmann *et al.*, 2016; Kingsolver & Buckley, 2017). Such phenotypic plasticity might be a contributing factor in the significance of environmental effects on specific components in this study. However, in general, our results based on trait means may underestimate the effect of plasticity or sex-specific variation in contributing to the overall assemblage trait distributions due to a lack of individual-specific morphological data.

Previous studies have revealed similar climatic niche conservatism in the thermal tolerance of tropical species, suggesting their limited ability to adapt to changing local environmental conditions through physiology (Grigg & Buckley, 2013; García-Robledo *et al.*, 2016). From our results, morphological traits such as body size and colour could be relevant to the thermal performance of organisms in addition to physiology. Moreover, the effects of morphology on the body temperature of ectotherms could be dependent on their thermoregulatory behaviour (Kingsolver, 1985; Bonebrake *et al.*, 2014). The interactive effects and co-adaptation across
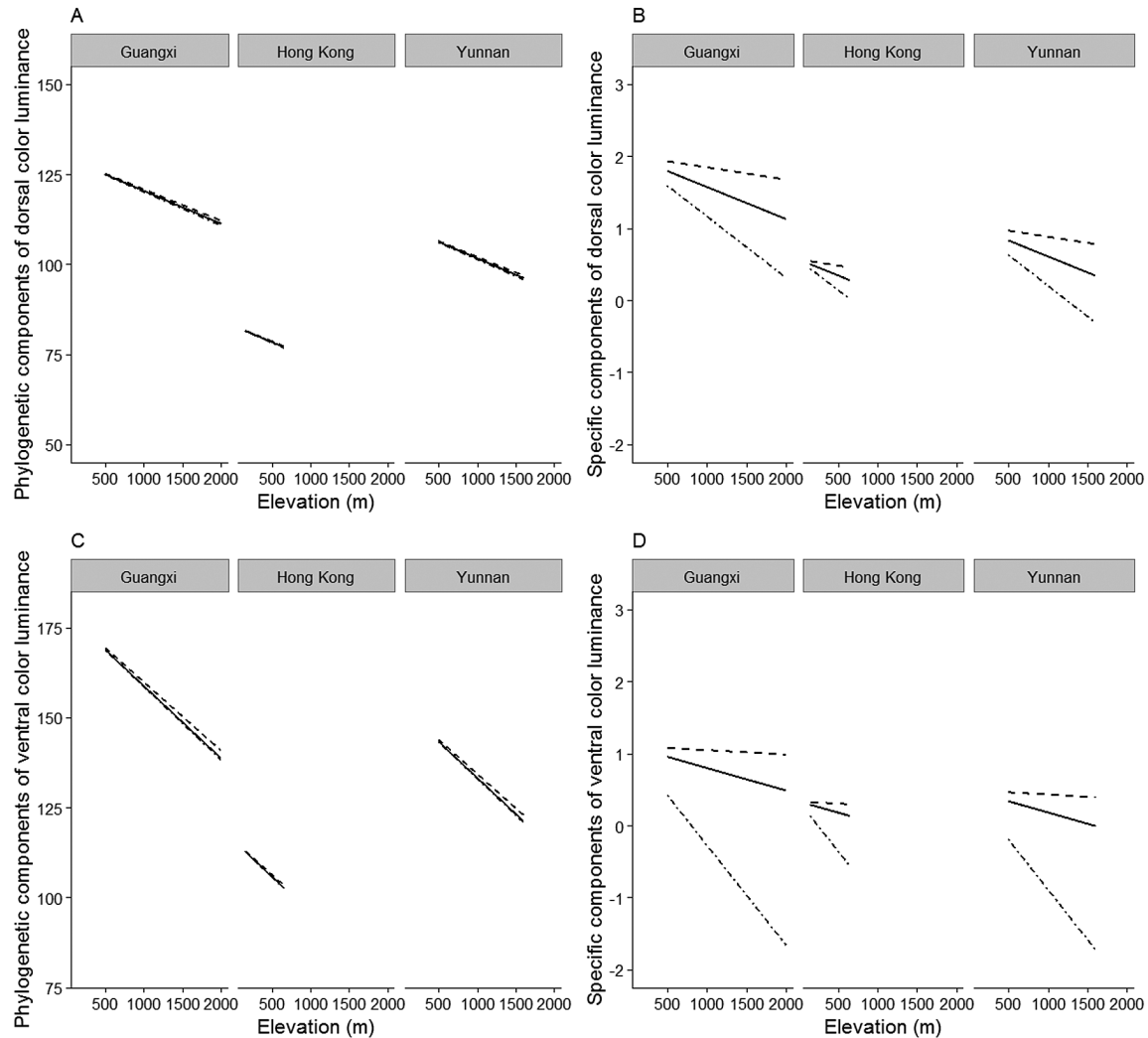
Figure 4. Modelled mean effects with smallest effects (dashed lines) and largest effects (dot-dashed lines) of elevation on phylogenetic (A, C)/specific (B, D) components of colour luminance for butterfly dorsal (A, B)/ventral (C, D) sides in three locations based on 300 phylogenetic trees.

physiology, morphology and behaviour can be complex, but are necessary in understanding why thermal stress may affect species through their traits (Huey & Bennett, 1987; Clusella-Trullas *et al.*, 2008; Peters *et al.*, 2016). Morphological and behavioural adaptation should be the targets of future study as these responses may mediate warming impacts on tropical species, which are nearer to their thermal upper limit and might be constrained to adapt to higher temperatures through physiological mechanisms (Araújo *et al.*, 2013; Bonebrake *et al.*, 2014; Sunday *et al.*, 2014).

In addition, biotic interactions such as sexual selection (Ellers & Boggs, 2003), predation and competition (Brown & Sax, 2004; Bonebrake, 2013; Cheng *et al.*, 2018) could also play important roles in shaping butterfly morphology, but these were not considered in this study. Multiple selection pressures, particularly

natural selection and sexual selection, may have trade-off relationships between different morphological functions. Within butterflies, wing melanism has advantages in cool climates but may not necessarily confer benefits in sexual selection (Ellers & Boggs, 2003), and could be constrained by nutrient resources during development (Talloen, Van Dyck & Lens, 2004). Moreover, other melanism functions such as disease resistance (Wilson *et al.*, 2001) and ultraviolet-B protection (Bastide *et al.*, 2014) may alter the elevation–colour luminance relationship from different selection processes, which might explain the insignificance of elevational effects on colour luminance at the species level. Therefore, morphological traits may not be able to easily evolve in one direction to adapt to singular changing environmental factors when other pressures exist, bringing costs to such adaptations (Ellers & Boggs, 2003).
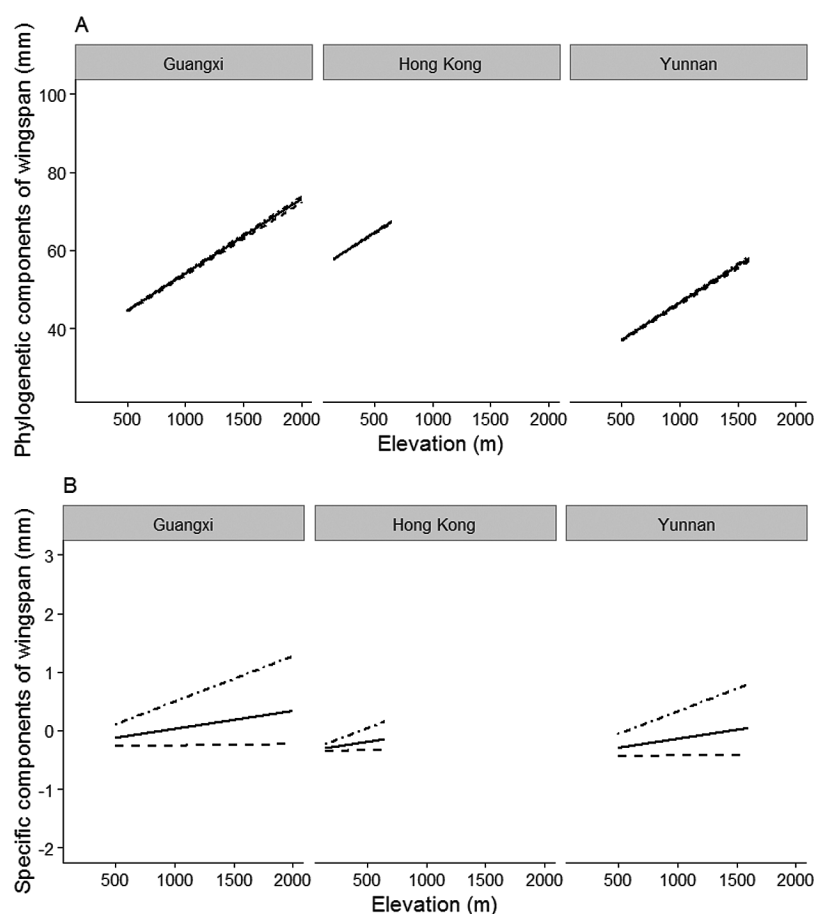
Figure 5. Modelled mean effects with smallest effects (dashed lines) and largest effects (dot-dashed lines) of elevation on phylogenetic (A)/specific (B) components of butterfly wingspan in three locations based on 300 phylogenetic trees.

In taking a synergistic approach including community assembly, morphological traits and phylogeny, we have demonstrated the role of elevation in shaping the distribution of butterflies through thermally relevant morphological traits in the tropics. The analysis also reveals the importance of evolutionary history in influencing morphological patterns and possibly constraining adaptive responses to climate change. Lastly, this study points to the vulnerability of tropical species when facing climate change and emphasizes the need for greater conservation research efforts on tropical climate change and biodiversity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Transect of each study site with temperature information from WorldClim during the breeding season (April to October) in 1970–2000.

Table S2. Names of sampled species with morphological information (N = 184 species).

Table S3. Five best multiple linear regression models for environmental factors and dorsal colour luminance of butterfly individuals.

Table S4. Five best multiple linear regression models for environmental factors and wingspan of butterfly individuals.

Table S5. Averaged statistical results of best multiple linear regression models for relationships of phylogenetic and specific components with environmental factors based on 300 phylogenetic trees.

Figure S1. The three study locations: Hong Kong (22°15′0″N, 114°10′0″E), Yunnan (XSBN: Xishuangbanna National Nature Reserve, 21°36′42″–58″N, 101°34′26″–47″E), Guangxi (MES: Mao'er Mountain Nature Reserve, 25°51′59.42″N, 110°24′46.05″E).

Figure S2. Hourly mean air temperature (A) and solar radiation (B) recorded along elevations at the three locations. Boxplots show medians with overall variances of temperature and solar radiation.

Figure S3. Bayesian 50% majority-rule consensus tree generated from ten gene regions (COI, COII, 16s, NADH1, NADH5, CAD, EF1α, GAPDH, IDH and *wingless*). Posterior probabilities are indicated at each node (PP). Among the species found in the sampling locations, we obtained sequences for 139 (out of 184 species recorded in this study) from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). Another 21 species of Lepidoptera were additionally selected for phylogenetic reconstruction: *Chersonesia rahria*, *Lamproptera meges*, *Mathoris loceusalis*, *Morova subfasciata*, *Potanthus flavus*, *Rhodoneura terminalis*, *Daimio tethys*, *Striglina cinnamomea*, *Hasora chromus*, *Hesperia comma*, *Danaus plexippus*, *Ocybadistes walkeri*, *Thymelicus lineola*, *Tagiades flesus*, *Pyrgus malvae*, *Coeliades forestan*, *Ctenoptilum vasava*, *Badamia exclamationis*, *Thyris fenestrella*, *Iphiclides podalirius* and *Polyura nepenthes*. Thus, in total we used 155 species of butterfly as in-group species and five species of moths as out-group species. We used mitochondrial gene regions (COI, COII, 16s, NADH1 and NADH5) and combined nuclear genes (CAD, EF1α, GAPDH, IDH and *wingless*). The sequences were aligned by MAFFT v.7.215 (Katoh & Standley, 2013). Sequences were concatenated and manually edited in Geneious v.4.8.5 (Kearse *et al*., 2012). The aligned concatenated matrix contained 10689 base pairs. Bayesian inference phylogenetic reconstruction was performed using MrBayes v.3.1.2. (Ronquist & Huelsenbeck, 2003). We used two independent MCMC chains each with 20 000 000 generations and sampled every 5000th generation. We set a cold Markov chain with the temperature parameter of 0.16, while keeping other priors as default. We adjusted the mean branch length prior to be 0.01 [brlenspr = unconstrained : exponential (100.0)] to reduce the likelihood of stochastic entrapment in local tree length optima (Brown *et al*., 2010; Marshall, 2010). The resultant standard deviation of split frequencies was < 0.03. The parameters sampled during the MCMC were checked by Tracer v.1.6 (Rambaut *et al*., 2014) and showed adequate effective sample sizes (ESS > 200). We excluded 1000 initial samples (around 25%) for each MCMC run (burn-in = 1000) from the summary analysis and calculated a 50% majority-rule consensus tree from the post burn-in trees. The posterior probabilities (PP) of each node were summarized (Larget & Simon, 1999) to infer support for individual clades: nodes with PP values ≥ 0.95 were considered well or strongly supported (Yang & Rannala, 1997). Since the incomplete gene information for some species caused relatively high uncertainty in phylogenetic reconstruction, we constrained the phylogenetic relationship with MrBayes according to previous high-level taxa of Lepidoptera phylogeny (Heikkilä *et al*., 2011; Regier *et al*., 2013).

Figure S4. Correlation between dorsal/ventral colour luminance and wingspan of 164 butterfly species.