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環境與系統發育限制對蛾類溫度耐受性和分佈的影響

Impacts of Environmental Conditions and Phylogenetic

Constraints on Moth Thermal Tolerances and

Distributions

許君咏

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本論文係許君咏 (R09247005) 在國立臺灣大學氣候變遷與永續發展國際學位學程完成之碩士學位論文，於民國 112 年 7 月 28 日承下列考試委員審查通過及口試及格，特此證明。

This master thesis is finished by Chun-Yung Hsu R09247005 at International Degree Program in Climate Change and Sustainable Development on 28 July, 112 of the Republic of China, passed the oral examination by the following examination committees.

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## 誌謝

這篇論文從 2016 在中橫收資料開始，後來到四川、馬來西亞採集，過程中有許多夥伴的努力及協助。

謝謝聖峰老師在我大三暑假，讓我有機會跟著團隊到馬來西亞採集，在研究所期間，每次和老師的討論也讓時常讓我走出迷霧。還要謝謝主導野外實驗的士緯，照顧我們兩個暑期生，也讓我們看見野外實驗的不確定性，以及應該用什麼態度應對，在論文撰寫期間也給我許多實質和精神上的支持，以及馬來西亞團隊的佩玲、賈媛、家軒，大家在旅途中的互相協助，開啟了我對生態研究的興趣。

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## 中文摘要

人為氣候變遷正在導致生物多樣性與生態系統的劇烈變動，更加凸顯深入了解物種對溫度變化反應的急迫性。本研究探討環境因素、演化過程和熱性狀之間複雜的交互作用，以及這些因素如何影響物種的分佈和對氣候變遷的韌性。我們在中國、臺灣和馬來西亞的山區進行了大規模的野外實驗，利用水浴槽實際測量蛾類的臨界溫度極限。我們在不同海拔高度設置 Robinson 式陷阱，收集物種的海拔分佈數據，並利用 iButton 記錄了當地的微氣候數據。我們使用系統發育比較方法和線性混合效應模型，以瞭解影響熱性狀和分佈的變量。與初始假設相反，我們發現環境條件比演化更能影響熱性狀和分佈，支持系統發育生態位保守主義 (phylogenetic niche conservatism) 的證據有限，這表示在決定蛾的溫度耐受性和分佈上，當前環境條件是比演化更關鍵的因素。我們也發現熱性狀和海拔分佈之間的顯著相關性，其中體型越大，溫度耐受範圍越窄。與經典的氣候變異度假說 (climate variability hypothesis) 相反，我們的研究結果顯示，在影響溫度耐受範圍的環境條件中，平均和極端環境溫度比整體氣候變異更為重要，這表示在快速變遷的氣候下，需要繼續研究這些複雜的關係，對於制定有效的保育策略，以及增加我們對物種在環境變化下大尺度生理反應的理解至關重要。

關鍵字：人為氣候變遷、熱性狀、海拔分布、系統發育生態位保守主義、氣候變異度假說



## ABSTRACT

Anthropogenic climate change is provoking substantial alterations in biodiversity and ecosystems, emphasizing the urgency for an in-depth understanding of species' responses to temperature fluctuations. This research delves into the intricate interaction between environmental elements, evolutionary processes, and species attributes, all of which play pivotal roles in determining species' distribution and resilience to climate change. We executed large-scale field investigations in the mountainous regions of China, Taiwan, and Malaysia, testing the critical thermal boundaries of moths using water baths. Elevation distribution data of species were gathered via Robinson's traps strategically placed along the elevation gradient, and iButtons were employed to document local microclimate data. To discern the critical variables affecting moth thermal characteristics and distribution, we utilized phylogenetically informed methodologies and linear mixed-effect models. Counter to our initial supposition, the data indicated that present environmental conditions exert a more considerable influence on moth thermal traits and distribution patterns than evolutionary lineage. The limited evidence we found for niche conservatism signifies a dominant role of current environmental conditions over hereditary traits in determining moth thermal tolerances and elevation distributions. Our research underscores notable associations between thermal tolerance range and moth distribution, with larger species showing more confined thermal tolerance ranges. Contrary to the classical climatic variability hypothesis, our study accentuates the critical influence of average and extreme environmental temperatures, more than overall climate variability, necessitating ongoing

exploration of these intricate relationships amidst rapidly evolving climates. The insights from our research are paramount in devising effective conservation strategies and broadening our comprehension of species' wide-scale physiological adaptations to environmental shifts.

Key words: Anthropogenic climate change, thermal traits, elevation distribution, phylogenetic niche conservatism, climatic variability hypothesis

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# 1.Introduction



The far-reaching effects of anthropogenic climate change have initiated profound shifts in biodiversity, ecosystem structure, and resilience across numerous regions. This includes geographic range shifts, alterations in the timing of seasonal events, and the vanishing of specialized ecosystems due to unprecedented conditions and dwindling thermal habitats propelled by global warming (IPCC, 2022).

Furthermore, temperature, a constant and pivotal influence on life, leaves its mark on organisms across various scales. From inciting chemical reaction rates at the molecular level (Hochachka & Somero, 2002) to steering ecological interactions at the ecosystem level (Dell et al., 2011), its role is undeniably critical. It shapes behaviors, dictates growth patterns, and ultimately shapes the adaptability and survival of species amidst a perpetually changing environment.

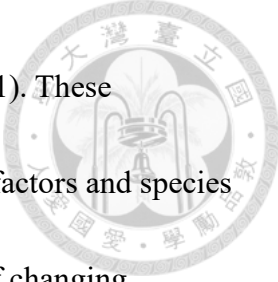
To accurately assess species' vulnerability in response to climate change, it is vital to delve into their physiological tolerance ranges, with a special emphasis on thermal tolerance. These ranges illuminate their fundamental niches, geographical distribution, and evolutionary dynamics, offering valuable insights into their resilience and adaptability amidst climate change (Gutiérrez-Pesquera et al., 2016; Hutchinson, 1981; Kearney & Porter, 2009). Given the current projections of escalating global

temperatures and increasing temperature variability (Parmesan & Yohe, 2003; IPCC, 2014), the understanding of how organisms endure extreme climatic conditions—both hot and cold—is an essential piece of the climate change puzzle.



The pivotal influence of environmental temperatures in shaping species distributions is well-documented (Parmesan, 2006). Factors like thermal tolerance and acclimation capabilities are considered crucial constituents of their physiological niche (Deutsch et al., 2008; Huey & Kingsolver, 1989; Somero, 2010). This growing curiosity about the evolution and operation of thermal limits has stimulated the formulation of a multitude of biogeographical hypotheses (Gaston et al., 2009).

The Climate Variability Hypothesis (CVH) theorizes that species residing in higher latitudes develop broader thermal tolerances. This adaptation enables these species to live across larger geographical regions and is believed to be a response to the more pronounced climatic variability and unpredictable weather patterns experienced in these areas, compared to regions closer to the equator (Chan et al., 2016; Ghalambor et al., 2006; Janzen, 1967). In contrast, the Environmental Filtering Hypothesis anticipates that in areas subject to high levels of abiotic stress, a strong environmental filtering effect occurs. This effect causes a more robust selection pressure on species' functional traits, promoting increased consistency of ecological traits among these species. This convergence in traits influences how species on the periphery of their ranges respond to



less favorable environmental conditions (Fischer, 1960; Wallace, 1891). These hypotheses emphasize the intricate interplay between environmental factors and species traits in shaping species' distribution and their resilience in the face of changing conditions.

The reasons why most species are confined to specific environmental conditions have always been a focal point in the fields of ecology, biogeography, and evolution (Darwin, 1859). In the face of accelerating climate change, this question gains even greater urgency. Accurate estimation of the environmental conditions that favor specific species is crucial for effective conservation strategies in a changing climate (IPCC, 2014; Mähn et al., 2023; Thomas et al., 2004). While some evidence suggests a connection between species' physiological tolerance levels and the extent of their geographical range, there has been a paucity of empirical studies exploring the link between physiological traits and variations in species' geographical distribution. More data from ecologically similar and related species are needed to deepen our understanding of the relationship between species' physiology and biogeography.

Despite advancements in our understanding of macroecological patterns, the elements influencing the relative geographic range sizes of organisms remain unclear. Alterations in range size could be spurred by a complex interplay of ecological and evolutionary processes, highlighting the need for further research to decipher these

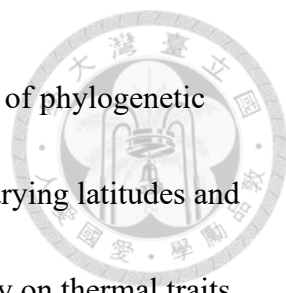


intricate dynamics.

Niche conservatism describes the propensity of species to maintain their ancestral ecological traits (Cooper et al., 2011; Grigg & Buckley, 2013; Kamilar & Cooper, 2013; Wiens & Graham, 2005). Consequently, closely related species might display analogous physiological traits, suggesting a limited capacity for adaptation (Losos, 2008).

Nevertheless, trait similarities between related or unrelated species could also relate to their shared experience of similar climatic conditions, and subsequent adaptations (Freckleton & Jetz, 2008). A species' geographical range reflects its historical and current environmental tolerances (Pearson & Dawson, 2003), with biogeographical and historical influences potentially leading to closely related species exhibiting divergent range sizes (Freckleton et al., 2002). These considerations underline the complex interplay of evolution, adaptation, and geography in determining species distribution.

To investigate the evolutionary constraints on physiological traits, we utilized comparative methods to assess phylogenetic signals. We employed Blomberg's  $K$  (Blomberg et al., 2003) and Pagel's lambda (Pagel, 1999)- popular tools in ecology used to measure phylogenetic signals in continuous traits. These methods were used to examine the thermal traits and distribution range of organisms. In exploring the relationships between organism traits (specifically thermal traits), their distribution, and environmental temperature, we leveraged Phylogenetic General Least Squares



(PGLS)(Martins et al., 2002). This method allows for the assessment of phylogenetic influence on data. We executed extensive field experiments across varying latitudes and altitudes to investigate the impact of climatic variables and phylogeny on thermal traits and organism distributions. Ultimately, our study aims to contribute valuable insights into macro-physiology, and how evolutionary processes and environmental factors shape the physiological traits and geographical distributions of species.

## 2. Materials and Methods

### 2.1 Study Areas and Species

Our study was conducted across three mountainous regions in East Asia, each at different latitudes: the Cameron Highlands in Malaysia (July 24 – August 21, 2019; 4°28'0"– 4°36'0"N, 101°11'0"– 101°23'0"E), the Central Mountain Crossing in Taiwan (July 20 – August 28, 2015; 24°16'0"– 24°21'0"N, 121°10'0"–121°40'0"E), and Jiajin Mountain in Sichuan, China (June 28 – July 22, 2017; 30°23'0"– 30°85'0"N, 102°69'0"– 102°90'0"E). These locations covered a broad altitude range, from 140 m to 1959 m above sea level (a.s.l) in Malaysia, 343 to 3140 m (a.s.l) in Taiwan, and 860 to 4150 m (a.s.l) in China. Our study focused on moths as they exhibit high species diversity and are particularly sensitive to ambient temperature. Furthermore, moths' phototropism, or attraction to light, makes them more readily collectible, enhancing the

feasibility of the study.



### **2.3 Estimating CT<sub>max</sub>, CT<sub>min</sub>, and thermal tolerance range**

Critical thermal tolerance is a fundamental functional trait that illustrates a species' adaptability to their environment (Bennett et al., 2018). While most prior studies on species' thermal tolerance were conducted under laboratory conditions (Overgaard et al., 2011; Pintanel et al., 2022), natural patterns of critical thermal minimum (CT<sub>min</sub>) and critical thermal maximum (CT<sub>max</sub>) across altitude and latitude are more likely to reflect a blend of genetic influences and plastic environmental responses (Shah et al., 2017). To capture this, we chose to measure thermal tolerance directly in the field, offering a more realistic representation of the physiological responses of individuals to varying environmental temperatures in their natural states.

We collected moths attracted by a 200-watt mercury light and white screen at night, along the altitude gradient in the three mountain regions. After random collection, the moths were placed in a sealed, transparent glass box (approximately 100\*80\*55 mm<sup>3</sup>) for 10 minutes. Once the moths were inactive, we recorded the individual's thoracic temperature using a thermal imaging camera. To test the upper and lower bounds of their thermal tolerance, we immersed the box in a water bath (50°C for heat

exposure and  $-4^{\circ}\text{C}$  for cold exposure). Each moth was subjected to only one condition.


When a moth reached its knockdown temperature, defined by the loss of muscle function coordination and inability to perch, it was immediately removed and its body temperature recorded with a thermal imaging camera (FLIR T420, FLIR Systems Inc., Danderyd, Sweden). In total, we tested 1475 individuals in Malaysia, 2257 in Taiwan, and 1917 in China. Voucher specimens were deposited in the Biodiversity Research Museum, Academia Sinica, Taipei, Taiwan.

Following the experiment, we used ThermaCAM Researcher Pro 2.10 software (FLIR Systems) to determine the thoracic temperature of each tested moth. We recorded the lowest and highest temperatures within the range as the critical thermal minimum (CT<sub>min</sub>) and critical thermal maximum (CT<sub>max</sub>) of each individual, respectively. The thermal tolerance range was then calculated as the difference between a species' CT<sub>min</sub> and CT<sub>max</sub>.

#### **2.4 Elevation distributions of moths**

We used modified Robinson-style traps with 15-watt UV lamps placed at roughly 250-meter elevation intervals in each mountain area to gather species' elevation distribution data. The sampling sites were strategically selected away from other artificial light sources, and areas where the forest composition remained undisturbed by






human activities. Traps were set in the afternoon and moths were collected the following morning, a process that was repeated at each sampling site. This methodology yielded 692 individuals in Malaysia, 3121 in Taiwan, and 984 in China. Voucher specimens were safely stored in the Biodiversity Researcher Museum, Academia Sinica, Taipei, Taiwan. The distribution data of the species subsequently identified in each mountain area was represented by the difference between the highest and lowest elevations where individual members of each species were found.

## **2.5 Climatic data determination**

To acquire detailed temperature data for each sampling site where the moth elevation traps were positioned, we utilized iButton devices. These compact electronic units were programmed to log temperature data at 30-minute intervals. For protection, each iButton was housed in a T-shaped plastic tube and suspended at a height of 1.5 meters above the ground level. Throughout the course of our experiment, these devices recorded daily maximum ( $T_{max}$ ) and minimum ( $T_{min}$ ) ambient temperatures. We then computed the mean ambient temperature ( $T_{mean}$ ) by averaging  $T_{max}$  and  $T_{min}$ , and calculated the diurnal temperature range (DTR) as the difference between  $T_{max}$  and  $T_{min}$ .

To assign the corresponding ambient temperature to each species, we pinpointed




the temperature data associated with the midpoint of the species' elevational range. If no temperature data directly corresponded with this midpoint elevation, we performed interpolation using the temperature measurements from the two nearest elevations. This approach allowed us to accurately estimate the ambient temperature experienced by each species.

## **2.6 Identification of moth species and morphological measurement**

The identification of individual moths down to the species level was a crucial part of this study. In instances where visual differences between similar taxa were not obvious, or when polymorphic individuals were difficult to identify based purely on appearance, we resorted to anatomical determination using genitalia. Our study encompassed a wide array of species: in Malaysia, we encountered 264 species spread across 34 subfamilies; in Taiwan, we identified 157 species within 33 subfamilies; and in China, we cataloged 232 species from 31 subfamilies. Each of these groups included one or more species for which we measured the critical thermal minimum (CT<sub>min</sub>) or critical thermal maximum (CT<sub>max</sub>).

Aside from species identification, we also represented body size through various measures, including dry weight, body length, and forewing length. To ensure the accuracy of these measurements and proper preservation of the specimens, we followed




a meticulous preparation process. The specimens were initially taken from storage at a temperature of  $-20^{\circ}\text{C}$ , then fitted with pre-measured insect pins, and finally dried in an oven set at  $45^{\circ}\text{C}$  for a period of five days. This method helped guarantee the integrity of the specimens and the precision of the morphometric measurements.

## **2.7 Phylogeny reconstruction**

For our phylogenetic analyses, we extracted DNA from the legs of moths collected in the field or from our sampling specimens. The extraction, amplification, and sequencing of DNA were conducted following the procedures outlined in previous research (Wahlberg & Wheat, 2008). We targeted one mitochondrial gene region (COI) and four nuclear gene regions (EF-1a-1, Rps5, GADPH, and Wingless) for our analyses. For those samples for which we were unable to extract DNA, we supplemented our data by downloading sequences from previous studies available on the online genetic sequence database, GenBank. These sequences were then analyzed in conjunction with our newly sequenced taxa to establish a comprehensive phylogenetic analysis of the moth species in our study. This combined approach allowed us to explore the genetic and evolutionary relationships among the different moth species, providing a broader context for our physiological and biogeographical observations.

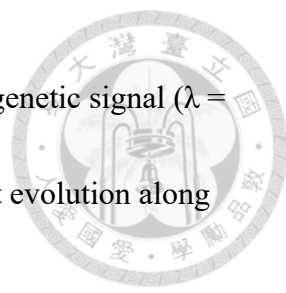
In our study, we reconstructed phylogenetic trees using both maximum likelihood



(ML) and Bayesian methods. We initially utilized the software jModeltest to perform ML analyses. Subsequently, we employed BEAST v.2.7 (Bouckaert et al., 2019) to simultaneously estimate a Bayesian topology and divergence times. Initially, BEAUti was used to configure the analysis parameters, including the model of evolution obtained in jModeltest (GTR). Based on nested sampling analyses, we applied an Optimized Relaxed Molecular Clock and birth-death tree model to achieve the best fit for our data. We then ran the resulting file using BEAST v.2.7 (Bouckaert et al., 2019) for 1,000,000,000 generations, starting from a random tree. To ensure adequate effective sample size (ESS) values and the convergence of our trees, we utilized Tracer v.1.7.2 (Rambaut et al., 2018). Finally, we generated a consensus tree using TreeAnnotator v.2.7.3 (Heled & Bouckaert, 2013), setting a burn-in of 100,000 states consistent with the burn-in observed in Tracer (Rambaut et al., 2018).

## **2.8 Testing for phylogenetic signal**

In order to examine the phylogenetic signal within each trait, we employed two commonly utilized metrics: Pagel's  $\lambda$  (Pagel, 1999) and Blomberg's K (Blomberg et al., 2003). Pagel's  $\lambda$  is a scaling parameter that measures the extent to which the covariance matrix among species for a specific trait is influenced by phylogenetic relatedness. We tested estimates of  $\lambda$  against two evolutionary hypotheses. The first hypothesis proposes




a star phylogeny in which all tips are equidistant, implying no phylogenetic signal ( $\lambda = 0$ ). The second hypothesis assumes a Brownian motion model of trait evolution along the given phylogeny ( $\lambda = 1$ ).

Blomberg's K is another metric used to evaluate phylogenetic signal, based on the premise of a Brownian motion (BM) model of trait evolution. In this model, trait evolution follows a random walk process, where the difference in trait values among species grows in direct proportion to the time since their shared ancestor, as denoted by the cumulative branch length from the root to each tip in the phylogenetic tree (Revell et al., 2008). A K value of 1 implies that trait evolution adheres to the predictions of Brownian motion. K values exceeding 1 suggest that taxa are more similar than anticipated under Brownian motion evolution, denoting a strong phylogenetic signal. Conversely, K values below 1 indicate greater trait divergence among taxa than projected by the Brownian motion evolution of the given phylogeny (Blomberg et al., 2003). These measurements provide insight into the extent to which evolutionary history has shaped the traits of the species in our study.

## **2.9 Phylogenetic generalized least squares (PGLS)**

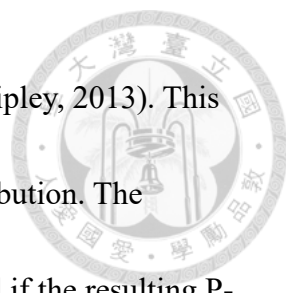
To investigate the correlation between thermal traits and geographical variables (elevation distribution of species), we employed the phylogenetic generalized least



squares (PGLS) models (Martins et al., 2002). These models were developed using a suite of R packages: 'caper' (Orme, 2023), 'ape' (Paradis et al., 2023), and 'phytools' (Revell, 2012) in R (version 4.12). We used a model selection procedure to discern the ecological traits that best explain the elevation distribution in our dataset, enabling us to examine different hypotheses effectively. This robust methodology offered a comprehensive understanding of the interplay between thermal traits and species' elevation distribution.

## **2.10 Relationship between elevation distribution range size, thermal traits, body size, and environmental temperature**

We conducted linear mixed-effects models to assess the associations between species thermal traits, elevational distribution range, and environmental temperatures. In these models, we considered species' subfamily identity and location as random effects. The development of these models was facilitated through the use of 'lme4' (Bates et al., 2015) and 'nlme' (Pinheiro & Bates, 2006) packages in R (version 4.1.2). To probe deeper into the direct and indirect influences on species thermal traits, we performed a confirmatory path analysis using a piecewise structural equation model (PSEM). This was achieved by applying a piecewise-fitted hierarchical linear mixed-effects model, a method facilitated by the 'piecewiseSEM' package (Lefcheck, 2016) in R. We evaluated




the overall path model using Shipley's test of directed separation (Shipley, 2013). This test generates a Fisher's C statistic that can be compared to a  $\chi^2$  distribution. The hypothesized causal relationship was deemed sufficiently reproduced if the resulting P-value was greater than 0.05. This comprehensive approach allowed for a detailed examination of the direct and indirect influences on species' thermal traits.

### 3. Results

We initially employed two prevalent metrics, Pagel's  $\lambda$  and Blomberg's K, to test for phylogenetic signals of traits at the subfamily level. It was found that the thermal traits (CTmax, CTmin, and thermal tolerance range) of moths from the three regions exhibited weak phylogenetic signals. The Pagel's  $\lambda$  values were small and associated with p-values all greater than 0.05, which suggests no significant difference from zero. Additionally, Blomberg's K values were below one with p-values exceeding 0.05 (Table 1). These results mirrored the observations for the elevation distribution range size across all three mountainous regions at differing latitudes. Additionally, when we investigated other morphological traits such as dry weight, body length, and wing length, the results consistently revealed weak phylogenetic signals among these traits.


Following this, we constructed a full model to conduct a phylogenetic generalized least square (PGLS) analysis, integrating thermal tolerance range, morphological traits



(dry weight, body length, and wing length), and environmental temperatures (ambient maximum temperature, ambient minimum temperature, diurnal temperature range) as predictors for the distribution range size. The analysis revealed that the thermal tolerance range was significantly correlated with the species elevation distribution range size (log-transformed) in both Malaysia and Taiwan (PGLS, Malaysia,  $P = 0.001$ ; Taiwan,  $P = 0.015$ , Table 2). Furthermore, a significant relationship was found between species dry weight and distribution range size in Malaysia ( $P = 0.021$ , Table 2). However, for the taxa in China, no significant correlation was detected between the thermal tolerance range and the distribution range size (PGLS,  $P = 0.168$ , Table 2). The environmental temperatures did not show any significant association with the altitudinal distribution range in any of the three mountain regions. Consistent with the results of the phylogenetic signal testing, the PGLS analysis also found no strong phylogenetic signals in the thermal traits range and distribution range size across all regions (Malaysia, Pagel's  $\lambda = 0$ ,  $P = 0.003$ ; Taiwan, Pagel's  $\lambda = 0$ ,  $P = 0.068$ ; China, Pagel's  $\lambda = 0$ ,  $P = 0.002$ , Table 2).


Additionally, we employed a linear regression model to investigate the impact of thermal traits on species distribution. Our results indicate a correlation between critical temperature and distribution range size (Figure 4a-b), suggesting that species with a higher tolerance for warm or cool temperatures exhibit wider distribution. Moreover, a





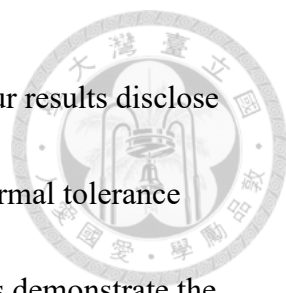
positive relationship was found between thermal tolerance range and distribution range size (Figure 4c). When considering the distribution limits, the species' critical maximum temperature showed a negative correlation with the distribution's lower limit (Figure 5a), while the critical minimum temperature also negatively correlated with the distribution's upper limit (Figure 5b).

To understand the relationships between various environmental temperature variables, we carried out a Principal Component Analysis (PCA). The PCA revealed that the first principal component (PC1) made a significant contribution, explaining 69% of the total variance, while the second principal component (PC2) accounted for 31% of the total variance, also a notable contribution (Figure 6). The factor loadings for PC1 showed strong correlations with variables like ambient maximum temperature (-0.56), ambient minimum temperature (-0.57), and average temperature (-0.59). In contrast, PC2 showed a significant relationship with the diurnal temperature range variable (0.90). This suggests that PC1 encapsulates overall temperature patterns, while PC2 emphasizes the variation within daily temperature. The component matrix further revealed the relationships between the original variables and these principal components. Altogether, the PCA results shed light on the underlying structure and variability in the environmental temperature dataset, thus providing a more nuanced understanding of the phenomenon under study.



Finally, we employed a Piecewise Structural Equation Model (PSEM) to incorporate both indirect and direct impacts of environmental temperature variables and moth body size on the thermal tolerance range and distribution ranges of moths. Our analysis revealed that PC1 exerted a significant influence on the thermal tolerance range (Figure 7a), whereas PC2 did not exhibit a substantial effect (Figure 7b). Importantly, dry weight showed a considerable impact on the thermal tolerance range, with larger body size individuals displaying a narrower thermal tolerance range. Moreover, thermal tolerance was found to have a profound effect on the elevation distribution range size at the subfamily level. Furthermore, we established that PC1, PC2, and dry weight have a significant effect on the critical thermal minimum (Table 4). This corresponds to our observation of larger body size individuals being less cold-tolerant as a result of their thermal tolerance range. Contrarily, neither PC1, PC2, nor dry weight influenced the critical thermal maximum (Table 4). This comprehensive model served as a platform for a more profound understanding of the complex interactions among various factors influencing the thermal tolerance and distribution range of moth species across different regions.

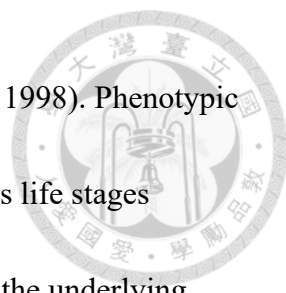
In conclusion, our study illuminates that the phylogenetic signals in thermal traits and distribution range size are not distinctively pronounced. However, we identified that the thermal traits of species still maintain a connection to their altitudinal distribution,



encompassing range sizes, upper limits, and lower limits. Notably, our results disclose that environmental temperature and moth body size influence the thermal tolerance range, and indirectly affect the distribution range size. These findings demonstrate the intricate interplay between thermal tolerance, morphological traits, environmental factors, and species distribution, enriching our understanding of the complexities underlying species adaptation to their environment.


## 4. Discussion

Contrary to our initial assumptions, environmental factors were observed to be more influential than phylogenetic determinants in explaining the variation in thermal traits and distributions patterns of moths. Our data indicate that the current environmental conditions exert a more substantial influential on the variation in thermal tolerances of moths than past evolutionary history, providing no support for a broader application of phylogenetic niche conservatism (Wiens & Donoghue, 2004). The existing literature offers a mixed perspective on the influence of niche conservatism on the thermal traits of ectotherms (Araújo et al., 2013; Mähner et al., 2023). However, our results align with the studies that found no evidence of climatic niche conservatism (Leal & Gunderson, 2012; Seebacher et al., 2015). Thermal traits of insects are attained




by phenotypic plasticity and evolutionary adaptation (Dahlggaard et al., 1998). Phenotypic plasticity has been noted in the thermal responses of insects at various life stages (Seebacher et al., 2015; Sgrò et al., 2016; Teder et al., 2022), though the underlying mechanisms may be intricate (Stillwell & Fox, 2005). Moreover, some studies suggests that genetic adaptation to high temperature may emerge from rapid evolution (González-Tokman et al., 2020; Skelly et al., 2007). Regarding moth distribution, our results do not lend support to the concept of niche conservatism, implying that phylogenetically related species do not necessarily share similar distribution patterns. This pattern could potentially be elucidated by the capabilities for dispersal and colonization inherent to these species. Many insects, particularly those with the capability of flight, are known to exhibit robust dispersal capabilities. This capability enables them to rapidly colonize new areas and habitats, which could account for the observed distribution patterns (Buckley & Kingsolver, 2012; Wiens & Graham, 2005).

Our results, as demonstrated in Figure 4, reveal a significant correlation between the elevational distribution of moth species and their thermal traits, including critical thermal maximum (CT<sub>max</sub>) and critical thermal minimum (CT<sub>min</sub>). In essence, the broader a species' thermal tolerance, the wider its distribution. Moreover, a positive correlation was discerned between thermal tolerance ranges and distribution range size, indicating that species with wider temperature tolerance can inhabit more extensive



ranges. Consequently, we observed that the upper elevational limit of species distribution expands with decreasing CT<sub>min</sub>, while the lower elevational limit contracts with increasing CT<sub>max</sub>. Hence, species possessing greater cold tolerance are equipped to occupy higher elevations, whereas species with enhanced heat tolerance are typically found in lower altitude regions. These findings corroborate previous studies suggesting that thermal physiology plays a pivotal role in determining species' distribution (Calosi et al., 2010; Olalla-Tárraga et al., 2011).

Our study unveils that the thermal tolerance range in insects is predominantly shaped by average and extreme environmental conditions, rather than by environmental variability. This assertion challenges the traditional interpretation of the Climate Variability Hypothesis (Janzen, 1967), which posits that species from more climatically variable environments should exhibit broader thermal tolerance ranges, resulting from their historical exposure to diverse conditions. Contrarily, our findings suggest that the significant influences on insects' thermal tolerance are not overall climatic variability, but specially average and extreme environmental conditions (Gaston & Chown, 1999). This elucidation implies that insects principally adapt to withstand conditions they encounter most frequently (average conditions), as well as the most intense, potentially life-threatening situations (Huey & Kingsolver, 1989). Infrequent temperature fluctuations might not exert substantial selective pressure to meaningfully shape thermal



tolerance (Kingsolver & Huey, 2008). Diving deeper into our analysis, we discovered that the critical thermal minimum (CT<sub>min</sub>) to be substantially impacted by both average and extreme environmental conditions. In stark contrast, we did not discern a definitive relationship between the critical thermal maximum (CT<sub>max</sub>) and environmental conditions.

A notable pattern was observed regarding body size: larger species demonstrated a narrower thermal tolerance range and were less tolerant of cold temperatures. These findings is consistent with previous research indicating that in ectotherms, among ectotherms - including insects, smaller-bodied species generally possess broader thermal tolerances (Addo-Bediako et al., 2000; Huey & Kingsolver, 1989). This wider tolerance could be attributed to their faster metabolic rates and superior heat dissipation in smaller individuals, allowing them to tolerate a wider range of temperatures (Forster et al., 2012). Concurrently, larger body sizes have been associated with narrower thermal safety margins, implying an increased vulnerability to extreme temperature events (Chown & Gaston, 2016; Sunday et al., 2010). Despite these patterns, we could not establish a distinct relationship between the critical thermal maximum (CT<sub>max</sub>) and body size, indicating that other variables not encompassed in our study may be affecting this trait. Furthermore, our findings underscore that dry weight exerts a more pronounced influence on thermal traits than environmental temperature across the three

studied locales. However, the mechanistic underpinnings of this relationship remain elusive, warranting more in-depth exploration in future research endeavors.



In our exploration of the intricate relationship between species lineage, environmental conditions, and thermal traits, with moths as our model organism, we discovered a compelling influence of average and extreme environmental temperatures on thermal traits, more important than phylogenetic factors. The correlation between moth morphology and thermal tolerance further underscored the pivotal role of environmental conditions. Our investigation, however, also highlighted areas that warrant further research. Future studies could benefit from considering additional environmental factors such as precipitation and habitat structures, which could also greatly affect these dynamics. While our work identified correlations between moth morphology and thermal tolerance, the effects of specific morphological traits on thermal physiology, and consequently on distribution patterns, necessitate more detailed scrutiny. Additionally, the rate and mechanisms of evolutionary adaptation of these traits remain a rich ground for future investigation. As our understanding of species distribution patterns under rapidly changing climates evolves, the importance of unraveling the interplay between thermal physiology and climatic adaptation becomes more evident. Hence, while our study has uncovered important insights, it underlines the necessity for ongoing, extensive research in this field.



## 5. Reference

- Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1445), 739–745.
- Angilletta, M. J., Huey, R. B., & Frazier, M. R. (2010). Thermodynamic Effects on Organismal Performance: Is Hotter Better? *Physiological and Biochemical Zoology*, 83(2), 197–206.
- Adopted, I. P. C. C. (2014). *Climate change 2014 synthesis report*. IPCC: Geneva, Switzerland, 1059-1072.
- Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., & Chown, S. L. (2013). Heat freezes niche evolution. *Ecology Letters*, 16(9), 1206–1219.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1 - 48.
- Bennett, J. M., Calosi, P., Clusella-Trullas, S., Martínez, B., Sunday, J., Algar, A. C., Araújo, M. B., Hawkins, B. A., Keith, S., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Villalobos, F., Ángel Olalla-Tárraga, M., & Morales-Castilla, I. (2018). GlobTherm, a global database on thermal tolerances for aquatic and terrestrial organisms. *Scientific Data*, 5(1), Article 1.
- Blomberg, S. P., Garland JR., T., & Ives, A. R. (2003). Testing for Phylogenetic Signal in Comparative Data: Behavioral Traits Are More Labile. *Evolution*, 57(4), 717–745.
- Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., Maio, N. D., Matschiner, M., Mendes, F. K.,





- Müller, N. F., Ogilvie, H. A., Plessis, L. du, Poppinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., ... Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLOS Computational Biology*, 15(4), e1006650.
- Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C., & Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, 79(1), 194–204.
- Chown, S. L., & Gaston, K. J. (2016). Macrophysiology – progress and prospects. *Functional Ecology*, 30(3), 330–344.
- Cooper, N., Freckleton, R. P., & Jetz, W. (2011). Phylogenetic conservatism of environmental niches in mammals. *Proceedings of the Royal Society B: Biological Sciences*, 278(1716), 2384–2391.
- Dahlgaard, J., Loeschke, V., Michalak, P., & Justesen, J. (1998). Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Functional Ecology*, 12(5), 786–793.
- Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection, Or, The Preservation of Favoured Races in the Struggle for Life*. J. Murray.
- Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C., & Mace, G. M. (2011). Beyond Predictions: Biodiversity Conservation in a Changing Climate. *Science*, 332(6025), 53–58.
- Dell, A. I., Pawar, S., & Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences*, 108(26), 10591–10596.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across



- latitude. *Proceedings of the National Academy of Sciences*, 105(18), 6668–6672.
- Fischer, A. G. (1960). Latitudinal Variations in Organic Diversity. *Evolution*, 14(1), 64–81.
- Forster, J., Hirst, A. G., & Atkinson, D. (2012). Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proceedings of the National Academy of Sciences*, 109(47), 19310–19314.
- Frazier, M. R., Huey, R. B., & Berrigan, D. (2006). Thermodynamics Constrains the Evolution of Insect Population Growth Rates: “Warmer Is Better.” *The American Naturalist*, 168(4), 512–520.
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic Analysis and Comparative Data: A Test and Review of Evidence. *The American Naturalist*, 160(6), 712–726.
- Freckleton, R. P., & Jetz, W. (2008). Space versus phylogeny: Disentangling phylogenetic and spatial signals in comparative data. *Proceedings of the Royal Society B: Biological Sciences*, 276(1654), 21–30.
- Gaston, K. J., & Chown, S. L. (1999). Elevation and Climatic Tolerance: A Test Using Dung Beetles. *Oikos*, 86(3), 584–590.
- Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., Clusella-Trullas, S., Ghalambor, C. K., Konarzewski, M., Peck, L. S., Porter, W. P., Pörtner, H. O., Rezende, E. L., Schulte, P. M., Spicer, J. I., Stillman, J. H., Terblanche, J. S., & van Kleunen, M. (2009). Macrophysiology: A Conceptual Reunification. *The American Naturalist*, 174(5), 595–612.
- Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen’s hypothesis revisited. *Integrative and Comparative Biology*, 46(1), 5–17.
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A., & Villalobos, F. (2020). Insect responses to heat: Physiological mechanisms,



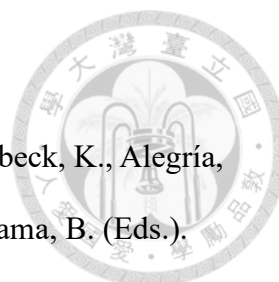
- evolution and ecological implications in a warming world. *Biological Reviews*, 95(3), 802–821.
- Grigg, J. W., & Buckley, L. B. (2013). Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. *Biology Letters*, 9(2), 20121056.
- Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. Á., Duarte, H., Nicieza, A., & Solé, M. (2016). Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography*, 43(6), 1166–1178.
- Heled, J., & Bouckaert, R. R. (2013). Looking for trees in the forest: Summary tree from posterior samples. *BMC Evolutionary Biology*, 13(1), 221.
- Hochachka, P. W., & Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press.
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, 4(5), 131–135.
- Hutchinson, G. E. (1981). *Introducción a la ecología de poblaciones*. Blume.
- Janzen, D. H. (1967). Why Mountain Passes are Higher in the Tropics. *The American Naturalist*, 101(919), 233–249.
- Kamilar, J. M., & Cooper, N. (2013). Phylogenetic signal in primate behaviour, ecology and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1618), 20120341.
- Kearney, M., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, 12(4), 334–350.
- Kingsolver, J., & Huey, R. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research*.
- Leal, M., & Gunderson, A. R. (2012). Rapid Change in the Thermal Tolerance of a Tropical



- Lizard. *The American Naturalist*, 180(6), 815–822.
- Lefcheck, J. S. (2016). *piecewiseSEM*: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7(5), 573–579.
- Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, 11(10), 995–1003.
- Mähn, L. A., Hof, C., Brandl, R., & Pinkert, S. (2023). Beyond latitude: Temperature, productivity and thermal niche conservatism drive global body size variation in Odonata. *Global Ecology and Biogeography*, 32(5), 656–667.
- Martins, E. P., Diniz-Filho, J. Alexandre F., & Housworth, E. A. (2002). ADAPTIVE CONSTRAINTS AND THE PHYLOGENETIC COMPARATIVE METHOD: A COMPUTER SIMULATION TEST. *Evolution*, 56(1), 1–13.
- Olalla-Tárraga, M. Á., McInnes, L., Bini, L. M., Diniz-Filho, J. A. F., Fritz, S. A., Hawkins, B. A., Hortal, J., Orme, C. D. L., Rahbek, C., Rodríguez, M. Á., & Purvis, A. (2011). Climatic niche conservatism and the evolutionary dynamics in species range boundaries: Global congruence across mammals and amphibians. *Journal of Biogeography*, 38(12), 2237–2247.
- Orme, D. (2023). The caper package: Comparative analysis of phylogenetics and evolution in R.
- Overgaard, J., Kristensen, T. N., Mitchell, K. A., & Hoffmann, A. A. (2011). Thermal Tolerance in Widespread and Tropical *Drosophila* Species: Does Phenotypic Plasticity Increase with Latitude? *The American Naturalist*, 178(S1), S80–S96.
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401(6756), Article 6756.
- Paradis, E., Blomberg, S. P., Bolker, B., Brown, J., Claude, J., Cuong, H. S., & Desper, R.

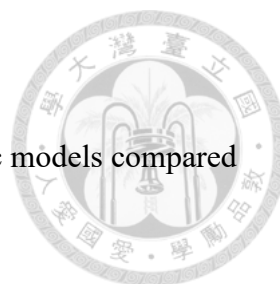


- (2023). Package ‘ape’. *Analyses of phylogenetics and evolution*. 2(4), 47.
- Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, 37(1), 637–669.
- Parmesan, C., Morecroft, M. D., Trisurat, Y., Adrian, R., Anshari, G. Z., Arneth, A., Gao, Q., Gonzalez, P., Harris, R., Price, J., Stevens, N., & Talukdar, G. H. (2022). Terrestrial and freshwater ecosystems and their services. In H.-O. Pörtner, D. C. Roberts, M. M. B. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Lösschke, V. Möller, A. Okem, & B. Rama (Eds.), *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.
- Parmesan, C., & Yohe, G. (2003a). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918), Article 6918.
- Parmesan, C., & Yohe, G. (2003b). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918), Article 6918.
- Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography*, 12(5), 361–371.
- Physiology, I. C. on C. (1987). *Comparative Physiology: Life in Water and on Land*. Springer Science & Business Media.
- Pinheiro, J., & Bates, D. (2006). *Mixed-Effects Models in S and S-PLUS*. Springer Science & Business Media.
- Pintanel, P., Tejedo, M., Merino-Viteri, A., Almeida-Reinoso, F., Salinas-Ivanenko, S., López-Rosero, A. C., Llorente, G. A., & Gutiérrez-Pesquera, L. M. (2022). Elevational and local climate variability predicts thermal breadth of mountain tropical tadpoles.



Ecography, 2022(5).

- Pörtner, H.-O., Roberts, D. C., Tignor, M. M. B., Poloczanska, E. S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., & Rama, B. (Eds.). (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.*
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, 67(5), 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 2, 217–223.
- Revell, L. J., Harmon, L. J., & Collar, D. C. (2008). Phylogenetic Signal, Evolutionary Process, and Rate. *Systematic Biology*, 57(4), 591–601.
- Seebacher, F., & Franklin, C. E. (2012). Determining environmental causes of biological effects: The need for a mechanistic physiological dimension in conservation biology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), 1607–1614.
- Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5(1), Article 1.
- Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What Can Plasticity Contribute to Insect Responses to Climate Change? *Annual Review of Entomology*, 61(1), 433–451.
- Shah, A. A., Gill, B. A., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., Kondratieff, B. C., Poff, N. L., Thomas, S. A., Zamudio, K. R., & Ghalambor, C. K. (2017). Climate variability predicts thermal limits of aquatic insects across elevation



- and latitude. *Functional Ecology*, 31(11), 2118–2127.
- Shipley, B. (2013). The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology*, 94(3), 560–564.
- Skelly, D. K., Joseph, L. N., Possingham, H. P., Freidenburg, L. K., Farrugia, T. J., Kinnison, M. T., & Hendry, A. P. (2007). Evolutionary Responses to Climate Change. *Conservation Biology*, 21(5), 1353–1355.
- Somero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers.’ *Journal of Experimental Biology*, 213(6), 912–920.
- Stillwell, R. C., & Fox, C. W. (2005). Complex Patterns of Phenotypic Plasticity: Interactive Effects of Temperature During Rearing and Oviposition. *Ecology*, 86(4), 924–934.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2010). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*, 278(1713), 1823–1830.
- Teder, T., Tait, K., Kaasik, A., & Tammaru, T. (2022). Limited sex differences in plastic responses suggest evolutionary conservatism of thermal reaction norms: A meta-analysis in insects. *Evolution Letters*, 6(6), 394–411.
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Townsend Peterson, A., Phillips, O. L., & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, 427(6970), Article 6970.
- Wahlberg, N., & Wheat, C. W. (2008). Genomic Outposts Serve the Phylogenomic Pioneers: Designing Novel Nuclear Markers for Genomic DNA Extractions of Lepidoptera. *Systematic Biology*, 57(2), 231–242.

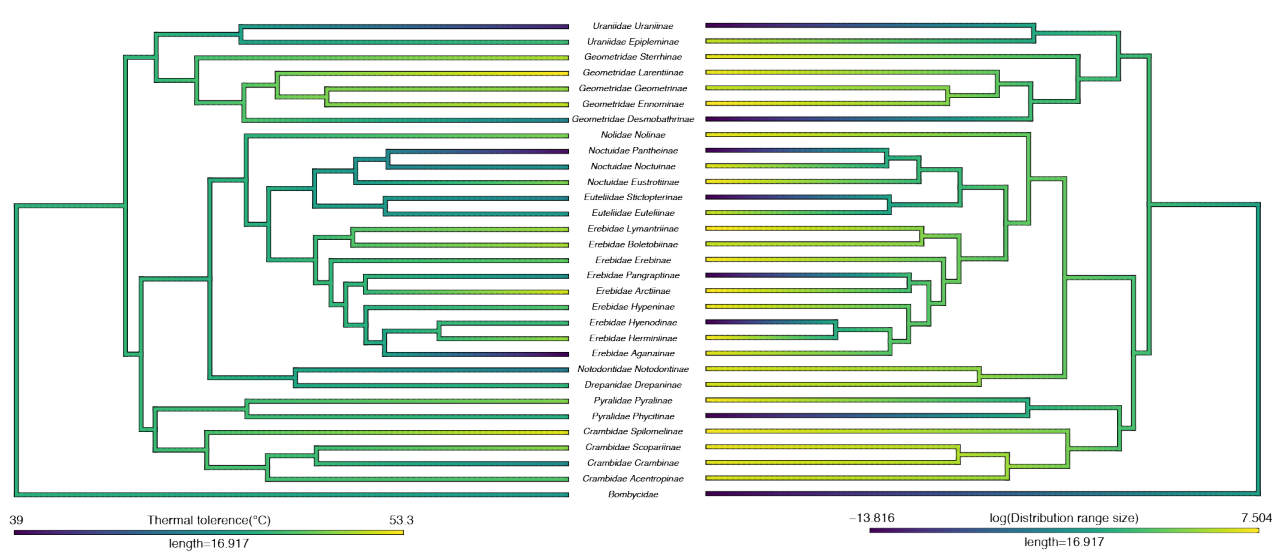


Wallace, A. R. (1891). *Natural Selection and Tropical Nature: Essays on Descriptive and Theoretical Biology*. Macmillan and Company.

Wiens, J. J., & Donoghue, M. J. (2004). Historical biogeography, ecology and species richness. *Trends in Ecology & Evolution*, 19(12), 639–644.

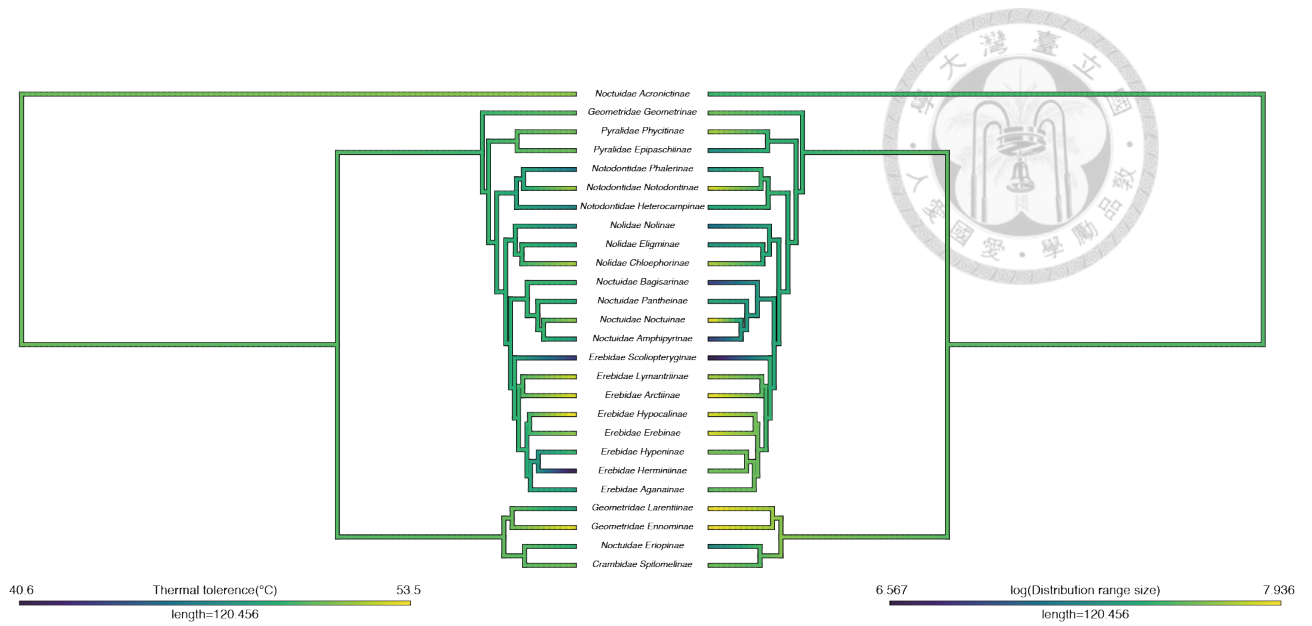
Wiens, J. J., & Graham, C. H. (2005). Niche Conservatism: Integrating Evolution, Ecology, and Conservation Biology. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 519–539.

## 6. Figures

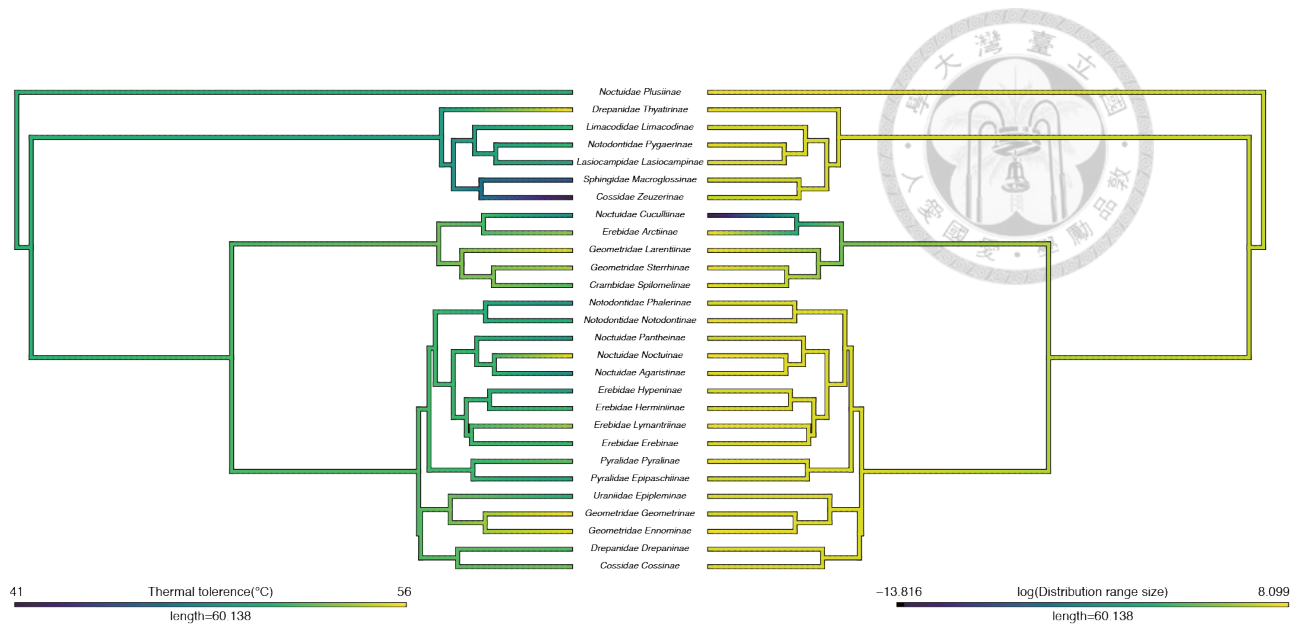


**Figure 1. Maximum likelihood (ML) ancestral state reconstruction of elevation distribution range and thermal tolerance range of Malaysia moths on a Bayesian tree from five gene region. The color gradient indicates the thermal tolerance range and elevation distribution range size with the widest (yellow) to narrowest (purple).**

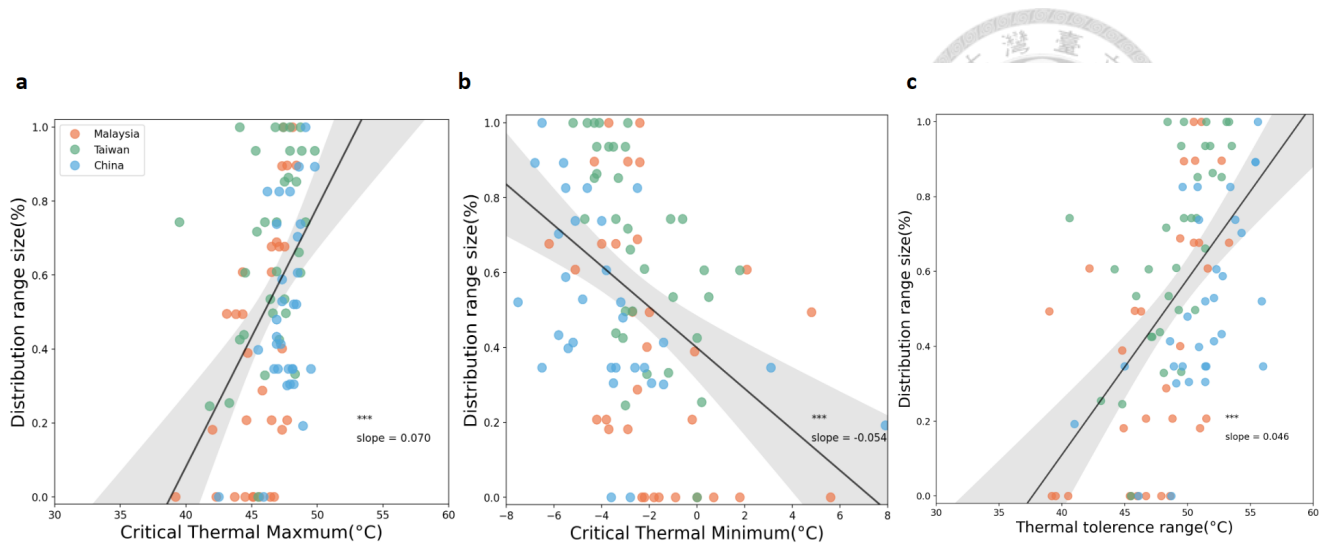




**Figure 2. Maximum likelihood (ML) ancestral state reconstruction of critical thermal tolerance range and thermal tolerance range of Taiwan moths on a Bayesian tree from five gene region. The color gradient indicates the thermal tolerance range and elevation distribution range size with the widest (yellow) to narrowest (purple).**



**Figure 3. Maximum likelihood (ML) ancestral state reconstruction of critical thermal tolerance range and thermal tolerance range of China moths on a Bayesian tree from five gene region. The color gradient indicates the thermal tolerance range and elevation distribution range size with the widest (yellow) to narrowest (purple).**



**Figure 4. Thermal tolerance traits of moths as a function of elevation distribution**

**range.** (a) Critical thermal maximum, (b) critical thermal minimum, and (c) thermal

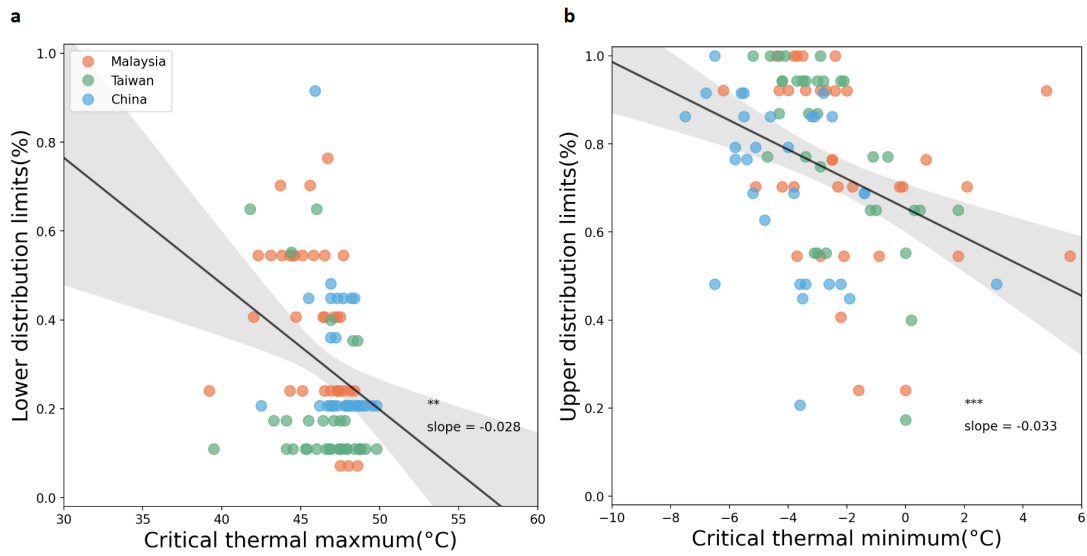
tolerance range in relation to distribution range size. The three locations are

distinguished by color (Malaysia, orange; Taiwan, green; China, blue.), solid lines are

significant relationships, dashed lines are insignificant relationships, shaded areas

represent the 95% confidence interval. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . For details,

see Table 3.



**Figure 5. Thermal tolerance limits of moths as a function of elevation distribution**

**limits.** (a) Critical thermal maximum in relation to lower distribution limit, and (b)

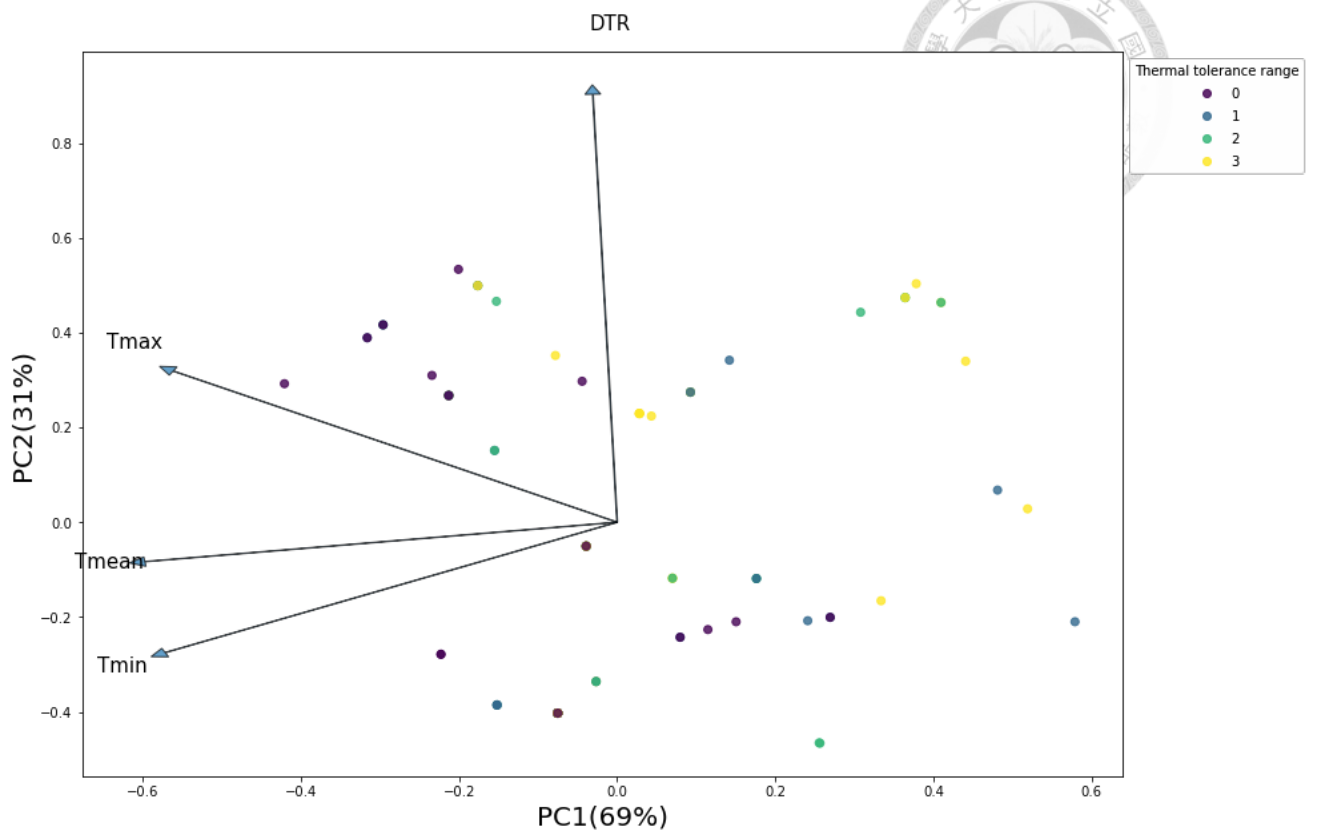
critical thermal minimum in relation to upper distribution limits. And the three locations

are distinguished by color (Malaysia, orange; Taiwan, green; China, blue.), solid lines

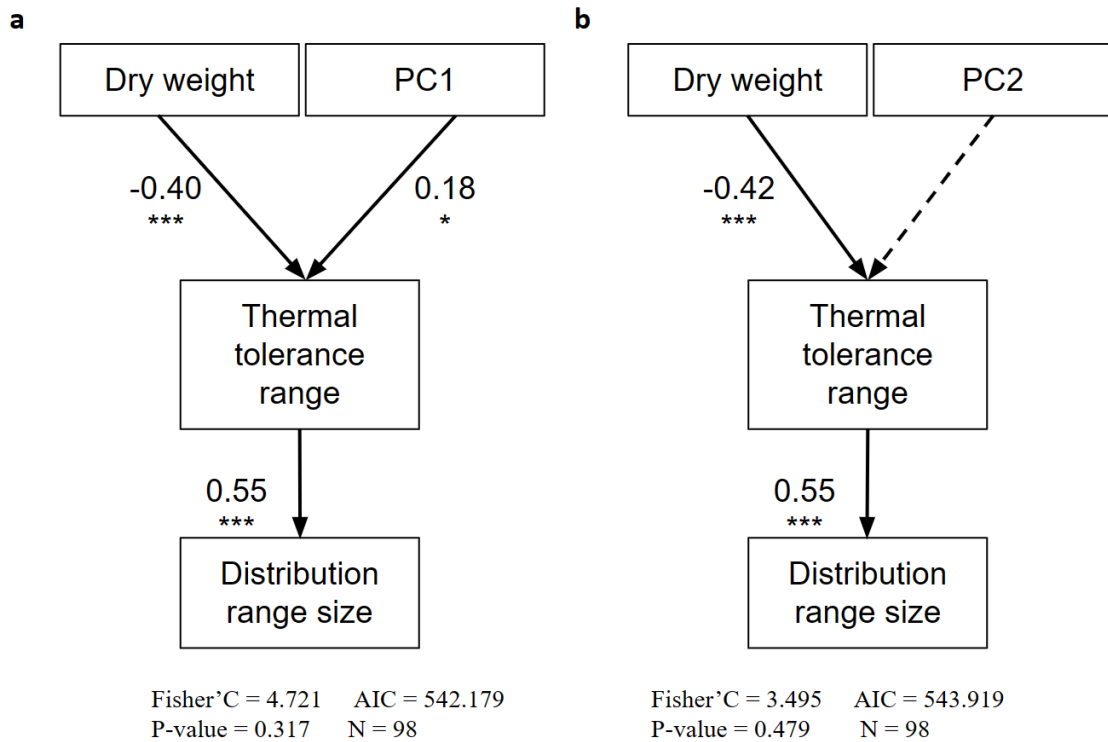
are significant relationships, dashed lines are insignificant relationships, shaded areas

represent the 95% confidence interval. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . For

details, see Table 3.



**Figure 6. Effect of mean environment and variability on thermal traits at subfamily levels.** Principal component analysis (PCA) of environmental variables. The color gradient indicates the thermal tolerance range with the widest (yellow) to narrowest (purple) of subfamilies in the variables space defined by the principal component axes PC 1 and 2.



**Figure 7. Distribution range size as a function of thermal tolerance, environmental variables, and specie morphology.** a-b Structural equation model of species dry weight and environmental variable principal components affecting the variation of species thermal tolerance range, and then affecting the distribution range size. Numbers next to arrows are estimates of standardized path coefficients (for details, see Table 4.) Solid lines are significant relationships, dashed lines are insignificant relationships. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

## 7. Tables



**Table 1. Phylogenetic signal test as Pagel's  $\lambda$  or Blomberg's K of moths' thermal traits, distribution range, and morphological traits.**

List of abbreviations: CTmax: critical thermal maximum; CTmin: critical thermal minimum; CTrange: thermal tolerance range; RS: elevation distribution range size; DW: dry weight; BL: body length; WL: wing length.

<b>Trait</b>	<b>Location</b>	<b>Pagel's <math>\lambda</math></b>	<b>P value</b>	<b>Blomberg' K</b>	<b>P value</b>
<b>CTmax</b>	Malaysia	0.000	1	0.734	0.272
	Taiwan	0.000	1	0.161047	0.919
	China	0.000	1	0.358	0.187
<b>CTmin</b>	Malaysia	0.000	1	0.605	0.826
	Taiwan	0.000	1	0.203	0.349
	China	0.182	0.401	0.396	0.096
<b>CTrange</b>	Malaysia	0.000	1	0.636	0.733
	Taiwan	0.000	1	0.182	0.792
	China	0.053	0.790	0.354	0.194
<b>RS</b>	Malaysia	0.000	1	0.653	0.638
	Taiwan	0.000	1	0.174	0.878
	China	0.000	1	0.292	0.393
<b>DW</b>	Malaysia	0.000	1	0.578	0.938
	Taiwan	0.000	1	0.233	0.344
	China	0.180	0.464	0.432	0.044
<b>BL</b>	Malaysia	0.000	1	0.602	0.876
	Taiwan	0.000	1	0.201	0.568
	China	0.023	0.905	0.360	0.196
<b>WL</b>	Malaysia	0.000	1	0.649	0.694
	Taiwan	0.000	1	0.199	0.527
	China	0.000	1	0.340	0.269

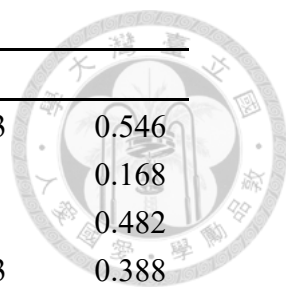


**Table 2. Phylogenetic generalized least squares for the analysis of distribution range size and thermal tolerance and morphology at subfamily level in three locations.**

Full model including all predictors and the response is the elevation distribution range sizes. List of abbreviations: CTrange: thermal tolerance range; DW: dry weight; BL: body length; WL: wing length; Tmean: mean ambient temperature; Tmin: temperature maximum; Tmin: temperature minimum; DTR: diurnal temperature range.

<b>Full model</b>					
<b>Location</b>	<b>Predictor</b>	<b>Estimate</b>	<b>SE</b>	<b>t value</b>	<b>P value</b>
<b>Malaysia</b> (n=31)	(Inceterpt)	-1588.734	2010.364	-0.790	0.437
	<b>CTrange</b>	72.560	20.341	3.567	<b>0.001**</b>
	<b>DW</b>	9.105	3.690	2.467	<b>0.021 *</b>
	BL	-19.132	16.237	-1.178	0.251
	WL	1.969	10.497	0.187	0.852
	Tmean	-1434.252	2187.473	-0.655	0.518
	Tmin	-1400.381	1856.956	-0.754	0.458
	Tmax	3441.736	4759.000	0.723	0.477
	DTR	-654.157	830.052	-0.788	0.439
<b><math>R^2 = 0.599</math>, p-value = 0.003**, <math>\lambda = 0</math>, kappa = 1.000, delta = 1.000</b>					
<b>Taiwan</b> (n=26)	(Inceterpt)	95.025	180.735	0.525	0.605
	<b>CTrange</b>	2.915	1.078	2.702	<b>0.015 *</b>
	DW	-0.255	0.181	-1.408	0.177
	BL	0.061	0.968	0.063	0.949
	WL	0.674	0.660	1.020	0.321
	Tmean	48.637	44.424	1.094	0.288
	Tmin	87.515	185.184	0.472	0.642
	Tmax	-187.475	305.725	-0.613	0.547
	DTR	51.901	91.105	0.569	0.576





$R^2 = 0.522$ , $p\text{-value} = 0.068$ , $\lambda = 0$ , $\kappa = 1.000$ , $\delta = 1.000$					
<b>China</b> (n=28)	(Intercept)	-65.263	106.303	-0.613	0.546
	CTrange	17.117	11.963	1.430	0.168
	DW	1.184	1.653	0.716	0.482
	BL	-5.736	6.493	-0.883	0.388
	WL	2.911	5.392	0.406	0.689
	Tmean	80.001	96.246	0.831	0.416
	Tmin	4.383	108.409	0.040	0.968
	Tmax	-107.858	142.460	-0.757	0.458
DTR	52.202	67.042	0.778	0.445	
$R^2 = 0.659$ , $p\text{-value} = 0.002^{**}$ , $\lambda = 0$ , $\kappa = 1.000$ , $\delta = 1.000$					

**Table 3. Linear mixed effects models for the analysis of thermal tolerance traits and distribution.**

The significance for the linear mixed effects models and variables was calculated using the F-test and t-test, respectively. List of abbreviations: CTmax: critical thermal maximum; CTmin: critical thermal minimum; CTrange: thermal tolerance range.

Response variable	Predictors	Estimate	SE	t value	P value	R <sup>2</sup>
Elevation distribution range size	Intercept	-2.572	0.642	-4.006	<0.001	0.609
	CTmax	0.066	0.013	4.840	<0.001	
	Intercept	0.377	0.082	4.593	<0.05	0.585
	CTmin	-0.052	0.011	-4.425	<0.001	
Lower distribution limit	Intercept	-1.861	0.377	-4.937	<0.001	0.632
	CTrange	0.048	0.007	6.481	<0.001	
Upper distribution limit	Intercept	1.282	0.349	3.667	<0.001	0.716
	CTmax	-0.020	0.007	-2.811	<0.01	
Lower distribution limit	Intercept	0.650	0.055	11.677	<0.001	0.704
	CTmin	-0.030	0.007	-4.136	<0.001	



**Table 4. Standardized parameter estimates for the structural equation models presented in Figure 8.**

List of abbreviations: PC1: principal component 1; PC2: principal component 2;

CTrange: Thermal tolerance range; DW: dry weight; RS: elevation distribution range size.

Response value	Predictors	Unstandardized			P value	Standardized Estimate (r)
		Estimate	SE	Critical ratio		
RS	CTrange	0.048	0.007	6.481	<0.001	0.554
CTrange	PC1	0.408	0.199	2.048	<0.05	0.182
	DW	-24.876	5.671	-4.386	<0.001	-0.400
RS	CTrange	0.048	0.007	6.481	<0.001	0.554
CTrange	PC2	-1.084	0.613	-1.769	0.080	-0.3216
	DW	-26.187	5.617	78.144	<0.001	-0.421
RS	CTmax	0.066	0.013	4.839	<0.001	0.418
CTmax	PC1	0.0089	0.126	0.070	0.9443	0.0072
	DW	-1.094	3.607	-0.303	0.762	-0.031
RS	CTmax	0.066	0.013	4.839	<0.001	0.418
CTmax	PC2	0.509	0.207	2.450	0.411	0.273
	DW	-0.712	3.651	-0.195	0.845	-0.020
RS	CTmin	-0.052	0.011	-4.424	<0.001	-0.410
CTmin	PC1	-0.414	0.123	-3.340	<0.01	-0.269
	DW	23.461	3.519	6.666	<0.001	0.551
RS	CTmin	-0.052	0.011	-4.424	<0.001	-0.410
CTmin	PC2	0.979	0.391	2.501	<0.05	0.424
	DW	24.541	3.565	6.882	<0.001	0.576