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ORIGINAL ARTICLE

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Examining elevation and thermoregulatory trait differences of endemic tropical swallowtail butterflies to assess their vulnerability to climate change

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Abstract

1. Tropical montane insects are vulnerable to climate change if up-hill range retractions reduce range sizes and increase extinction risk. Endemic species will be particularly at risk if they disproportionately occur at higher elevations, although threats may be ameliorated if they have morphological traits associated with improved thermoregulatory ability. We examine the vulnerability of endemic swallowtail butterflies on Sulawesi (Indonesia) to climate change by quantifying inter-specific variation in traits associated with thermoregulation (body size and melanism) and whether species' traits are associated with the elevation of species' ranges.

2. We collected data on size, melanism and elevation of 29 swallowtail butterfly species (Lepidoptera: Papilionidae) from 754 specimens at the Natural History Museum (United Kingdom) and the Zoologische Staatssammlung München (Germany).

3. Endemic butterflies were less melanised (16 endemic species, mean = 83% melanised; 13 non-endemic species = 89% melanised) but similar to non-endemics in size (endemic, mean = 56 mm; non-endemic = 53 mm) and elevation (endemic, mean = 345 m.a.s.l.; non-endemic: 291 m). There was no evidence that species size or melanism patterns were related to elevation in either group.

4. Few differences in elevation of endemic and non-endemic species imply that endemic swallowtail butterflies are not disproportionately more threatened by climate-driven mountaintop extirpation than non-endemic species. Further work is needed to examine whether paler wings alter the ability of endemic species to thermoregulate and adapt to changing climates. In the absence of reductions in anthropogenic global greenhouse gas emissions, on-going climate warming will continue to threaten tropical montane species.

KEYWORDS
elevation, endemism, Lepidoptera, morphology, museum collections, Papilionidae, Sulawesi

INTRODUCTION

Climate change and habitat loss are having unprecedented effects on biodiversity globally (Díaz et al., 2019), with global mean temperatures increasing by 1.1°C since pre-industrial times (Gulev et al., 2021). Tropical species are particularly vulnerable to these threats because they are often adapted to a narrower range of temperatures, living much closer to their thermal limits (Bonebrake, 2013; Sheldon, 2019). Thus tropical species may be disproportionately threatened by climatic warming (Deutsch et al., 2008), as well as from the high rates of forest loss and conversion to agriculture (Phalan et al., 2013). These threats are concerning given that tropical ecosystems support very high species richness, including many endemic species (Myers et al., 2000). Thus, studies are urgently needed to investigate the vulnerability of tropical endemic species to climate change.

Endemic species (i.e., species with small ranges, restricted to a particular location globally) may be especially at risk from climate change if they disproportionately occur at higher elevations (Kier et al., 2009; Manes et al., 2021; Myers et al., 2000). Endemics can be restricted to higher elevations due to past macroecological or biogeographical processes (Caterino & Recuero, 2024; Steinbauer et al., 2016; Suzuki et al., 2023). For example, montane regions provide climate refugia, leading to reduced extinction rates during past glacial–interglacial climate events (Harrison & Noss, 2017; Loarie et al., 2009; Monroe et al., 2022). The subsequent isolation of high-elevation populations on mountaintops is likely to have reduced gene flow (Flantua et al., 2020), increasing the potential for allopatric speciation (Flantua et al., 2020). Concentrations of endemics in montane regions may also reflect taxon cycle evolutionary theories (Wilson, 1961), with species retracting to higher elevations following the arrival of new colonists (Ricklefs, 2011; Ricklefs & Bermingham, 2002) or loss of lowland natural habitats (Jones et al., 1987). Montane species often lack opportunities for climate-driven up-hill shifts, which could result in endemics being vulnerable to extirpation (or extinction) at mountaintops (e.g., Minter et al., 2020). However, the evolutionary history of the tropical montane endemics might also influence their capacity to respond to anthropogenic warming in situ, for example, if they have persisted in the same locations during warmer interglacial periods, when they would have had to withstand hotter or drier conditions, and this can be tested using morphological traits associated with thermoregulation. Body size and wing melanism are two traits that are closely associated with thermoregulation in insects (Chown & Gaston, 2010; Kingsolver, 1983; True, 2003).

Bergmann's rule proposes that species living at cooler locations should be larger (Bergmann, 1848; Goldenberg et al., 2022; He et al., 2023). Although more often applied to endothermic species, these temperature–size patterns have been observed in insects (Atkinson, 1994; Hodkinson, 2005). However, there are complex relationships between body size, net radiation balance and convective heat transfer in insects (Gates, 2003; Kingsolver, 1983). Comparisons of body size across elevational gradients have revealed little consensus (Dillon et al., 2006; Horne et al., 2018; Shelomi, 2012), with

studies finding positive elevation–size relationships (e.g., Xing et al., 2018), no relationships (e.g., Haga & Rossi, 2016) and negative relationships (e.g., Baranovská & Knapp, 2017). This lack of consensus may be due to differential temperature–size responses in large and small-bodied insects (Tseng et al., 2018) but may also reflect the importance of other evolutionary drivers of body size, such as predation risk (Chown & Gaston, 2010). In addition, studies have also found insect size changes associated with warming (e.g Bowden et al., 2015; Fenberg et al., 2016).

Thermoregulation in insects is also affected by melanism (Álamo et al., 2023; Bogert, 1949; Goldenberg et al., 2022; Kang et al., 2021). Darker individuals can warm up faster than paler individuals (Gates, 2003; Kingsolver, 1983; Xing et al., 2016), improving fitness in cooler locations (Clusella-Trullas & Nielsen, 2020; Ellers & Boggs, 2004; Roland, 1982; True, 2003). Thus, positive elevation–melanism relationships are common in ectotherms (Clusella-Trullas et al., 2008), with more melanised individuals found at higher elevations (e.g., Lepidoptera: Gautam & Kunte, 2020; Coleoptera: Koslov et al., 2021; Hymenoptera: Bishop et al., 2016). The distribution of melanism on an insect's body is also important (Wasserthal, 1975; Álamo et al., 2023), with basal (i.e., close to the body) wing melanism associated with greater flight activity and higher body temperature in *Parnassius phoebus* F. (Lepidoptera: Papilionidae), but no effect of distal wing melanism (Guppy, 1986). However, melanism patterns may not be linked to thermoregulation but instead reflect disease resistance, wing structural strength (Dubovskiy et al., 2013), sexual signalling (Wiernasz, 1989), crypsis (e.g., Gloger's rule, where melanism is suggested to increase with humidity for better camouflage; Delhey, 2019) and/or aposematism (Majerus, 1998), and so insects occurring at high elevations may not necessarily be darker.

Sulawesi (Indonesia) is a highly topographically diverse island (maximum elevation is 3440 m.a.s.l., see Figure S1) and is a biodiversity hotspot harbouring exceptional levels of endemism (Whitten et al., 2002), with over 40% of butterflies endemic to the Sulawesi Region (c.f. 2% endemism in the Malay Peninsula, Vane-Wright & de Jong, 2003). These exceptional levels of endemism are also seen in other Sulawesi taxa (mammals: 61%; birds: 34%; and reptiles: 26%, Whitten et al., 2002). The formation of the Makassar Strait and subsequent isolation of Sulawesi from the mid-Eocene (~42 million years ago) onwards was a key driver for this exceptional endemism (Moss & Wilson, 1998; Vane-Wright & de Jong, 2003). As a consequence, Sulawesi has received considerable interest resulting in a long history of specimen collecting by entomologists, including butterflies (e.g., Martin, 1914; Wallace, 1869). Museum collections are large repositories of biodiversity information (Meineke et al., 2018; Pyke & Ehrlich, 2010) that have been exploited to examine changes in biodiversity patterns (e.g., Brooks et al., 2016; Jönsson et al., 2021) and provide a large volume of data on tropical species that are not available otherwise.

We use the collections at the Natural History Museum in London, UK (NHMUK) and the Zoologische Staatssammlung München in Munich, Germany (ZSM), which have significant numbers of butterflies collected from Sulawesi. In this study, we focus on Sulawesi

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swallowtail (Lepidoptera: Papilionidae) butterflies because they are a well-represented group in museum scientific collections (with thousands of specimens collected since the 1820s). There are approximately equal numbers of endemic and non-endemic Sulawesi swallowtail species (16 of 34 species of Sulawesi swallowtails are endemic), and they represent around 6% of the world's swallowtail butterflies (Vane-Wright & de Jong, 2003). We use museum material to examine the vulnerability of tropical butterflies to anthropogenic climate change, investigating morphological variation in endemic and non-endemic species. We test the hypotheses that, compared with non-endemic species, endemic species (1) occur at higher elevations and (2) are larger and darker, with the expectation that melanism is concentrated in the central part of the butterfly if it is associated with thermoregulation. We also examine (3) whether elevation–size and elevation–melanism relationships differ between endemic and non-endemic species, which would imply different sensitivities to climate.

METHODS

Data sources

We obtained information from museum specimens held at the NHMUK and the ZSM. Of the 34 Papilionidae species recorded to occur on Sulawesi (Vane-Wright & de Jong, 2003), representatives of 29 were found in the collections and included in our study (Table S1). A total of 16 of these species are endemic to Sulawesi (Vane-Wright & de Jong, 2003). We analysed male specimens only because female specimens are much less well-represented in museum collections (Cooper et al., 2019; Table S1), with disproportionally more female butterfly specimens lacking locality information. We excluded any specimens that were damaged. This resulted in 1042 male specimens potentially available for analysis (1–95 individuals per 29 species), but not all specimens had detailed locality information, preventing the determination of elevation (see below).

Determining location and elevation of specimens

For each specimen, we extracted collection locality from the label and georeferenced it to point coordinates using '*Gazetteer: Celebes*' (United States Hydrographic Office, 1944). Where the named locality was not found, we then searched for the location using Nominatim OpenStreetMap (http://nominatim.openstreetmap.org/). Where the localities were still not found, we searched associated literature and The Fuzzy Gazetteer (http://isodp.hof-university.de/fuzzyg/query/) to identify alternative spellings or modern variants of the specified locality name. Location data were obtained for the Indonesia administrative divisions of 'city' (area of 163–388 km^2) or 'regency' (1151–1844 km^2) or at finer spatial scales (e.g., 'village' [<5 km^2] or 'district' [46–1132 km^2, Database of Global Administrative Areas, https://gadm.org/]), and georeferenced coordinates represent the centre point of the location. For each specimen, we also extracted elevation

data from the specimen label where available. Where elevation was not specified, we estimated the elevation of the locality based on the georeferenced coordinates. We used de Ferranti Shuttle Radar Topography Mission Digital Elevation Model (http://www.viewfinderpanoramas.org/dem3.html; 3 arc second (90 m) resolution) and extracted the elevation of each georeferenced coordinate using the R package *raster* (Hijmans, 2022) in R Studio (R Core Team, 2022). Of the 1042 specimens, 288 specimens did not have detailed locality information, resulting in analyses of 754 specimens from 29 species (1–75 specimens per species).

Imaging and measuring butterfly size and colour morphology

We imaged the butterflies using an Olympus EM-5 Mark II mirrorless camera, with either an Olympus 60 mm Macro Lens or an Olympus 14-150 mm Telephoto Lens, through the Olympus Capture software (https://app.olympus-imaging.com/olympuscapture/en/). Each specimen was placed in a customised specimen tray and imaged with a scale bar and colour checker. The camera was mounted on a copy stand and a light box, with a ring bulb used as the light source (see Supplementary Material for more details, Figure S2a).

To quantify differences in butterfly size, we measured the length of both the right and left forewing (Figure S2b) from the images, using ImageJ (https://imagej.nih.gov/ij/). Forewing length is a well-preserved trait in pinned Lepidoptera specimens and strongly correlated with overall body size (García-Barros, 2015). We examined the repeatability of measures by re-measuring specimens from five species of the genus *Papilio* L. (*P. ascalaphus* B., *P. blumei* B., *P. fuscus* G., *P. jordani* F. and *P. sataspes* F. & F.). We measured one specimen per species a total of 10 times, and measurements were within 2% (mean difference = 1.36 mm) revealing that our methods for quantifying size were robust (Table S2).

To quantify differences in melanism (% black of dorsal body), we carried out three steps (Figure 1). First, we corrected the white-balance of the images using the colour checker and the macro Chart_-White_Balance plugin on the ImageJ software (https://imagejdocu.list.lu/plugin/color/chart_white_balance/start) to account for differences in lighting conditions when imaging specimens. Then, we cropped the image to the extent of the butterfly using the 'scissors select' tool in GIMP 2.8 (The GIMP Development Team, 2019). We then set the threshold of red, green and blue (RGB) values that represented the extent of 'black' colouration on the wing for all study species. To determine an appropriate threshold, we ran all the cropped images through RGB colour thresholds at intervals of 0.1 from 0 (completely black) to 1 (completely white), in the R package *countcolors* (Weller, 2019). This revealed that determining the threshold was relatively consistent and straightforward across all species, based on visual inspection of Figure S3 and our focal threshold sensitivity analyses (see Supplementary Material for more details). The threshold at which the melanism value was least sensitive was 0.6, such that varying the threshold by 0.1 around the selected threshold of 0.6 only
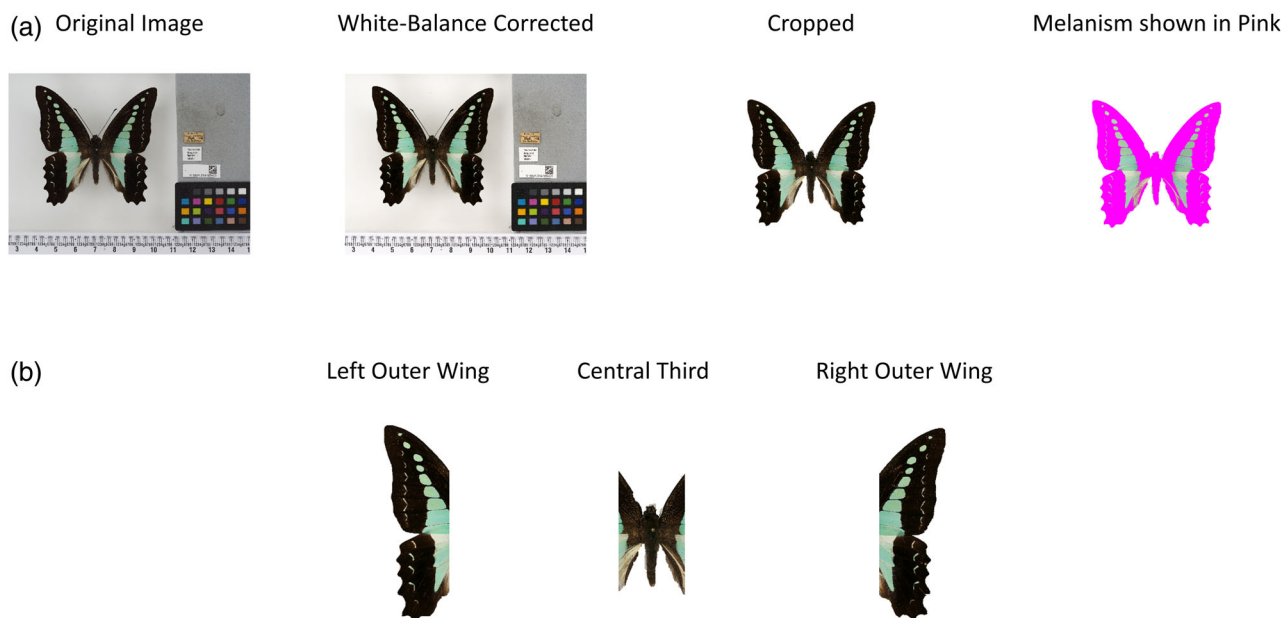
FIGURE 1 An example specimen to show steps taken to (a) calculate melanism (% black of dorsal body) value and (b) split butterfly into thirds (see main text for details).

changed the proportion melanised by 0.04%. We set 0.6 as our threshold, with all values below the threshold designated as 'black' and computed the percentage area of the wing that was black as our measure of melanism.

To examine the position of melanism on the butterfly body, we divided specimen images into three equal sections (left outer wing, central third and right outer wing; Figure 1b) using the R package *magick* (Ooms, 2021) and calculated melanism values for each of the three sections using the same methods as described above. We compared the central melanism value with the arithmetic mean of the left and right outer wing measures.

Data analysis

To test the hypotheses that endemic species occupy higher elevations, and are larger and darker than non-endemic species, we built three generalised linear mixed effects models, with elevation (m.a.s.l.), forewing length (in millimetres) and melanism (% black of dorsal body) as dependent variables, endemism as a fixed effect and species nested within genus as categorical random effects. To test the hypotheses that endemic species have different elevation–size and elevation–melanism relationships to non-endemic species, we built two more generalised linear mixed effects models, with forewing length (in millimetres) and melanism (% black of dorsal body) as dependent variables, elevation (m.a.s.l.), endemism, and their two-way interaction as fixed effects and species nested within genus as categorical random effects. We accounted for phylogenetic relatedness using random effects because there is no published phylogeny that includes all species included in this study. We implemented a backwards

stepwise elimination procedure, sequentially removing the least significant variable using *p*-values from *F*-tests until all remaining variables were significant with $p < 0.05$. We always removed non-significant interactive effects ($p > 0.05$) before the removal of the main effect. We also tested for differences in size and melanism between endemic and non-endemic species using the full set of male specimens ($N = 1042$) and found that the results were consistent with our analyses run using only male specimens with locality data ($N = 754$, analyses reported in the Supplementary Material).

To test the hypothesis that melanism was higher in the centre of the butterfly compared with the outer wing region, we built a generalised linear mixed effects model, with melanism (% black) as the dependent variable, position (i.e., centre or outer wing), endemism and their two-way interaction as fixed effects and specimen ID nested within species nested within genus as categorical random effects. Where there were significant differences, we conducted a pairwise Tukey post hoc least-squares means comparison to identify the groups that differed from each other.

We built these generalised linear mixed models in R Studio (R Core Team, 2022) using the R packages *broom.mixed* (Bolker & Robinson, 2022), *car* (Fox & Weisberg, 2019), *lme4* (Bates et al., 2015), *MASS* (Venables & Ripley, 2002) and *MuMIn* (Barton, 2022). We used the *lsmeans* R package (Lenth, 2016) to conduct our post hoc testing. We plotted results using the R package *ggplot2* (Wickham, 2016).

The swallowtail *Lamproptera meges* Z. (Green Dragontail) is the only representative of the genus *Lamproptera* G. that occurs in Sulawesi and is much smaller than other Papilionidae species included in our analyses and is therefore an outlier in our analyses. Thus, we reran all our models with and without *L. meges* included (26 specimens), but

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TABLE 1 Total number of Sulawesi (Indonesia) swallowtail butterfly (Papilionidae) male specimens analysed.

	All specimens	Specimens with precise locality information to village level	Specimens with precise locality information to district level	Specimens with precise locality information to city/regency level
No. specimens	1042 (555)	206 (99)	600 (317)	754 (407)
No. species	29 (16)	27 (15)	29 (16)	29 (16)
Mean no. individuals per species	35.9 (34.7)	7.6 (6.6)	20.7 (19.8)	26 (25.4)

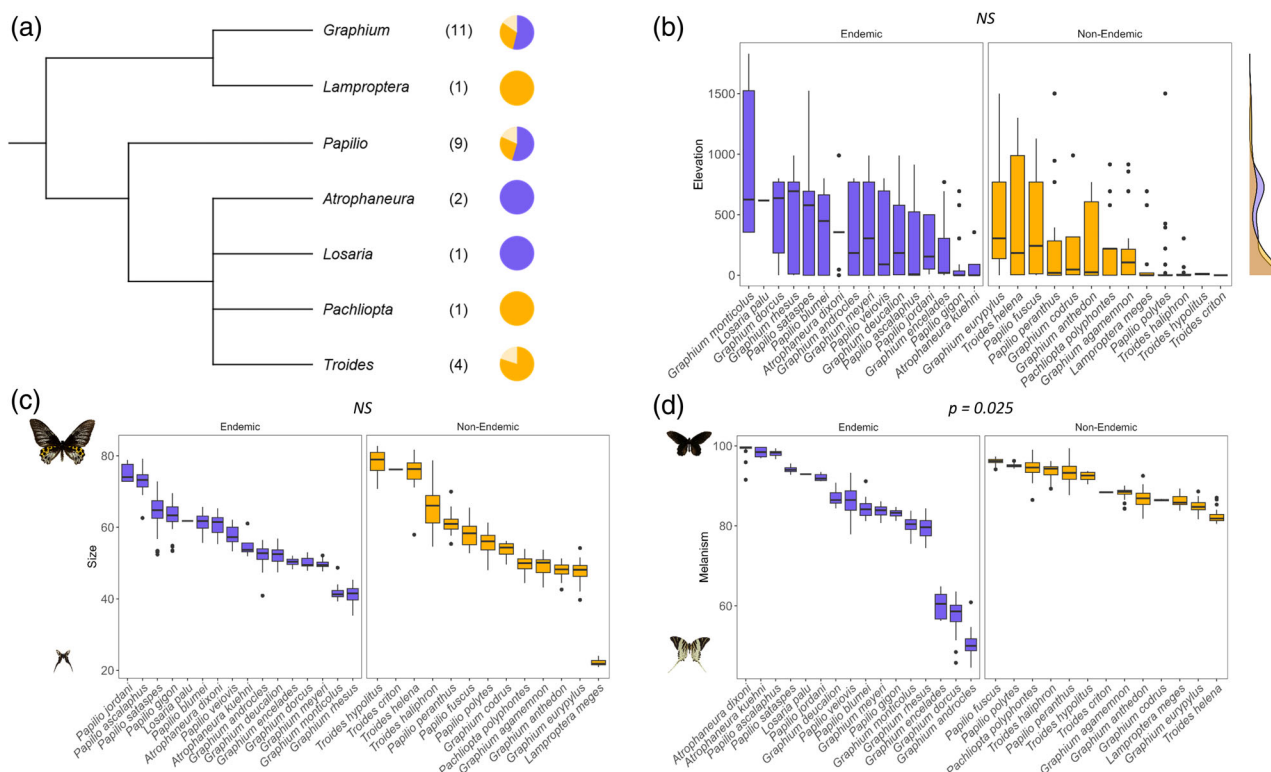*Note*: Numbers of endemic specimens are in parentheses.

FIGURE 2 Summary information for 29 swallowtail butterfly species (endemic = purple shading; non-endemic = yellow). Phylogenetic relationships in (a) are based on Simonsen et al. (2011), with all genera included within the Troidini clade placed together. The pie charts in (a) are based on Vane-Wright and de Jong (2003), with light yellow shading representing five Sulawesi species without data that were not included. The number of species in each genus included in our study is in brackets. Boxplots show median and interquartile ranges for species by (b) elevation (m.a.s.l.), (c) size (forewing length, mm) and (d) melanism (% black of dorsal body). The marginal density plots in (b) show the distribution intensity of endemic and non-endemic species across elevations. *p*-values indicate whether endemic and non-endemic butterflies differed and *NS* indicates no significant difference. Images of butterflies have been shown for reference.

our findings were broadly the same, and so we report data for the full data set in the main article. We report all the analyses without *L. meges* in the Supplementary Material.

RESULTS

Sulawesi butterflies

We found 1042 specimens of male swallowtail butterflies from Sulawesi in museum collections (Table 1). The specimens had been

collected over a period of at least 170 years, with the oldest specimen collected around 1820 and the most recent specimen collected in 1997. Specimens were mainly collected before 1940 (Figure S5), and peak collecting periods are associated with the major European collectors: G van der Capellen (1815–1826), AR Wallace (1856–1859), H Frühstorfer (1895–1896), W Doherty (1896) and L Martin (1906–1907). This pattern reflects differences in specimen collecting over time, with specimen collecting being very popular in the 19th and early 20th century, but tighter collecting restrictions more recently reduced the number of modern specimens, with none from the 21st century. Clear hotspots of specimen collection are around Makassar,
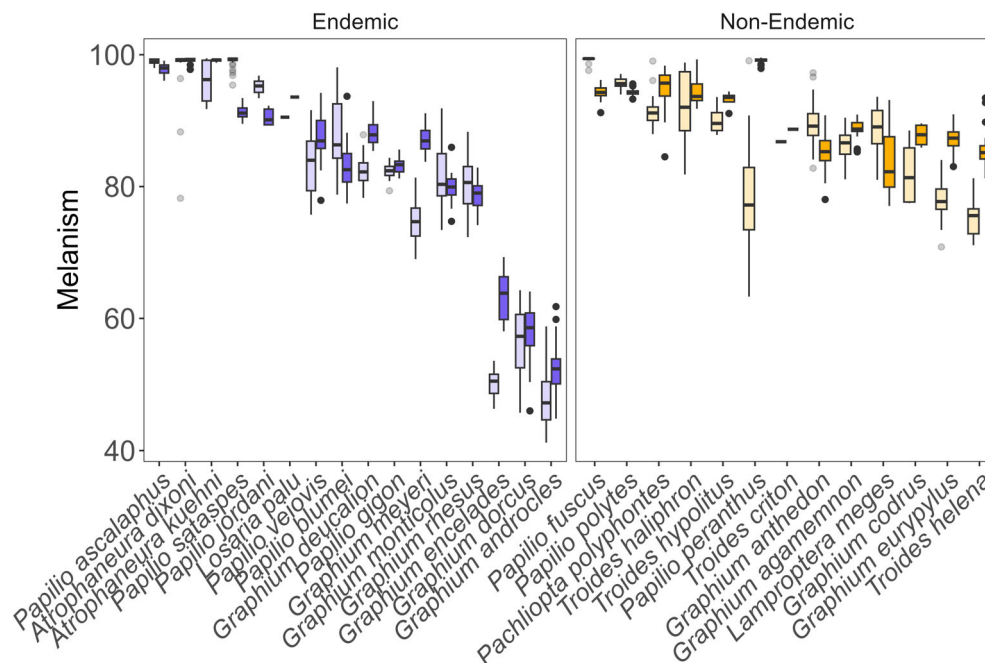
FIGURE 3 Boxplots (median and interquartile ranges) showing the distribution of melanism (% black) on 29 swallowtail butterfly species (endemic = purple shading; non-endemic = yellow) according to occurrence on the central part of the body ('centre', i.e., body and inner wing; light shading) compared with outer areas ('outer wing', mean of left and right outer wing; dark shading; see main text for details). Melanism was significantly lower in the centre than the wings for both endemic and non-endemic butterflies ($p < 0.001$).

Manado and Palu (Figure S1), which reflects the locations of key ports of Sulawesi.

We analysed 754 specimens from 29 species, of which 16 species (55%; n = 556 specimens) were endemic (Figure 2a). The species varied markedly in elevation (0 to 1829m, Figure 2b), size (forewing length: 20.9 to 82.8 mm, Figure 2c), melanism (% black of dorsal body: 44.5 to 99.8%, Figure 2d) and elevation (0 to 1829 m, Figure 2d).

Comparing endemic and non-endemic species

Contrary to expectation, we found no significant difference in the elevation of collection points for endemic (mean ± SE = 345 ± 18 m) and non-endemic (mean ± SE = 291 ± 22 m) species ($\chi^2 = 1.493$, df = 1, $p = 0.222$, Figure 2b) and no difference in variation (Levene's test: $F = 0.644$, df = 1, $p = 0.423$). We also found no difference in size between endemics (mean ± SE = 55.8 ± 0.49 mm) and non-endemics (mean ± SE = 52.8 ± 0.67 mm; $\chi^2 = 1.23$, df = 1, $p = 0.267$, Figure 2c) or variation in size (Levene's test: $F = 2.542$, df = 1, $p = 0.111$).

We found that endemic butterflies were significantly (8.77%) less melanised (mean ± SE = 81.7 ± 0.71% melanised) than non-endemic butterflies (mean ± SE = 89 ± 0.26% melanised, $\chi^2 = 5.03$, df = 1, $p = 0.025$, Figure 2d). Endemic butterflies were also significantly more variable in melanism (i.e., % black of dorsal body) than the non-endemic butterflies (Levene's Test: $F = 121.7$, df = 1, $p < 0.001$). We found that melanism was significantly greater in the outer wing region

than the central region for both endemic (centre = 80.5% melanised; outer wing = 82.1%; Tukey post hoc test, $p < 0.001$) and non-endemic butterflies (centre = 86.3% melanised; outer wing = 90.5%; Tukey post hoc test $p < 0.001$; $\chi^2 = 166.0$, df = 3, $p < 0.001$, Figure 3). This position of melanism on the butterfly body indicates that melanism is probably not primarily associated with thermoregulation in these species. We did not find any elevation–size ($\chi^2 = 2.16$, df = 3, $p = 0.539$, Figure 4a) or elevation–melanism relationships ($\chi^2 = 5.44$, df = 3, $p = 0.143$, Figure 4b).

DISCUSSION

The 29 species of Sulawesi swallowtail butterflies that we studied varied considerably in the elevation where they were collected, in size and in melanism. Unexpectedly, endemic species were less melanised but did not differ in size or elevation compared with non-endemic species. We also found no elevation–size or elevation–melanism relationships in either endemic or non-endemic species. Taken together, these findings suggest that temperature is likely not the key driver of size and melanism variation in the Sulawesi swallowtail butterflies.

Elevation

Our findings that endemic species do not occupy higher elevations than non-endemic species contrasts with findings from other studies
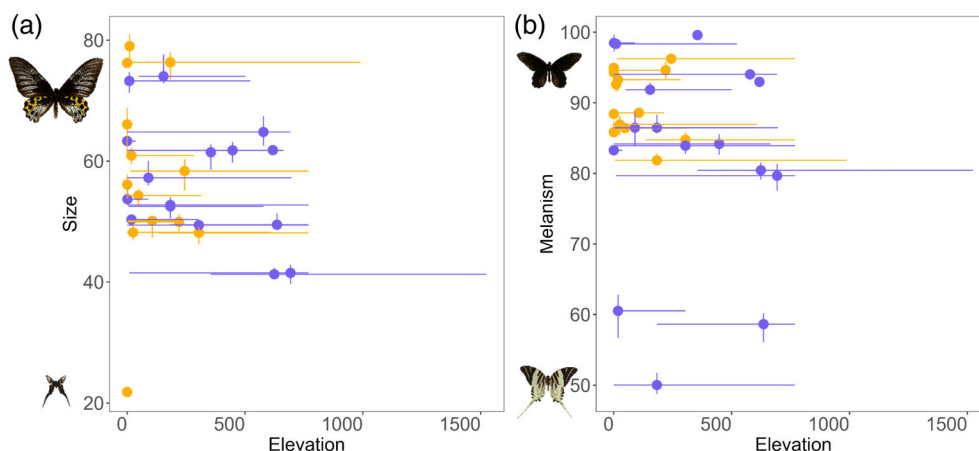
FIGURE 4 Relationships between elevation (m.a.s.l.) and (a) size (forewing length, mm) and (b) melanism (% black of dorsal body) in Sulawesi swallowtail butterflies. Data points are median values per species (N = 29 species; 16 endemic = purple, 13 non-endemic = yellow), with the error bars representing the upper and lower quantiles. There were no significant relationships between elevation and size, or between elevation and melanism. The butterfly images are shown for reference.

of tropical butterfly communities (e.g., Lewis et al., 1998). Despite a 54 m difference in the mean elevation between endemic and non-endemic species in the expected direction, this mean difference is very small relative to the extremely large variation in elevation across both groups (Figure 2b). Most of the specimens in the museum collections are from localities in the lowlands, which correspond with what is known of collectors' journeys (e.g., Wallace, 1869). Only 27.3% (206/754 specimens) of location information was at a fine spatial scale ('village' (<5 km^2), finer), and so some of these findings may reflect a lack of precision in the elevation information extracted from specimen label data or simply reflect relatively limited elevational range within the sampling effort of collectors. However, we found no difference in the spatial resolution of information for endemic and non-endemic species (Table 1; e.g., specimens specific to village-level: endemic = 13.1%; non-endemic = 14.2%), implying the lack of any differences between endemics and non-endemics was not due to resolution biases in label information.

We found that specimens from most endemic species were found and collected at low elevations, although many of these localities on Sulawesi have had on-going forest loss for more than 150 years (Martin, 1914; Wallace, 1869). Major deforestation occurred around major ports of Sulawesi (e.g., Makassar and Manado; Figure S1), which were important sites for butterfly collection (Martin, 1914; Wallace, 1869), such that by 1950 (when most of the specimens sampled had already been collected), most of these areas were largely deforested (Hannibal, 1950; Wallace, 1869). Present-day occurrence data from the Global Biodiversity Information Facility (https://www.gbif.org/, e.g., iNaturalist records) and modern day accounts of the study species (e.g., Nakae, 2021) also indicate that many endemic species continue to be found at lowland locations (see analyses in Supplementary Material). Endemic butterfly species are likely to be forest-dependent species (Cleary & Mooers, 2006; Lewis et al., 1998; Wurz et al., 2022), implying that they would have been collected from

historically modified lowland areas where there was rainforest remaining within the landscape.

Lewis et al. (1998) suggest that endemic butterflies on Grande Comore may occur at higher elevations than non-endemics because endemic species are more confined to forests, which have been lost at low elevations. By contrast, our results suggest that some of the endemic butterflies of Sulawesi can occur in modified habitats, given that forests have also been lost at low elevations on Sulawesi. Some Sulawesi swallowtail butterflies have larval host plants that occur in urban and agricultural areas or secondary forests (Igarashi & Fukuda, 1996, 2000; Igarashi & Harada, 2015). For example, some of our study species, including endemics, are known to feed on *Citrus* trees and cultivated Rutaceae as larvae (Igarashi & Fukuda, 1996, 2000; Igarashi & Harada, 2015; Robinson et al., 2023; Vane-Wright & de Jong, 2003), and citrus trees were commonly planted in urban areas in Sulawesi during the 1850s (Wallace, 1869). Other studies have also shown that endemic butterflies can occur in human-modified landscapes (e.g., Wurz et al., 2022). Occurrence of endemics in lowland areas on Sulawesi may reflect the very long history of people modifying landscapes in these regions (Whitten et al., 2002), as well as species habitat associations, although other endemic species are restricted to natural forest habitats (e.g., Fermon et al., 2005).

Our results suggest that endemism does not necessarily increase with elevation (c.f. Steinbauer et al., 2016). However, we note that some endemic swallowtail species (*Losaria palu* and *Graphium monticolus*) occurred only at higher elevations (355–1828.8 m, Figure 2b) and five endemic species (*G. monticolus*, *G. dorcus*, *G. rhesus*, *L. palu* and *Papilio sataspes*) had median elevations greater than 500 m, which were patterns not seen in non-endemic species. Our findings suggest that endemic Sulawesi swallowtail butterflies occur at similar elevations to non-endemics and so may not be disproportionately vulnerable to mountaintop extirpation through up-hill climate-driven distribution shifts. Nonetheless, montane species are threatened by

climate change (Mata-Gruel et al., 2023), and species dependent on closed-canopy forest will also be threatened by land-use change (Liseki & Vane-Wright, 2011).

Morphological traits

We found no differences in the sizes of endemic and non-endemic species on Sulawesi. Butterflies on Sulawesi have been observed to be larger than close relatives on neighbouring islands (e.g., Borneo), with 'gigantism' conclusions suggested by Wallace (1869). However, this 'gigantism' does not apparently differ among endemics and non-endemics, or in relation to elevation (proxy for temperature; Figure 4a), suggesting that other factors drive gigantism on Sulawesi (Foster, 1964).

Our initial hypothesis was that endemic species would be larger and darker because we expected them to occur at higher (i.e., cooler) elevations, compared with non-endemic species. However, not only do we find no differences in size or elevation but we also find that endemic butterflies are less melanised than non-endemic butterflies. This difference is mainly due to an absence of pale non-endemic species (Figure 2c). We find no elevation–melanism patterns in our study, which indicates that temperature is unlikely to be the key driver of melanism patterns (Zeuss et al., 2014), but instead may be due to other factors, such as sexual signalling (True, 2003), wing structural strength or protection from pathogens (e.g., Dubovskiy et al., 2013) or ultraviolet irradiation (Katoh et al., 2018). The lack of any relationship with temperature or thermoregulation is supported by our finding that melanism is not concentrated around the body of the butterflies (Figure 3).

Museum collections

We used museum specimens to test for differences in elevation, melanism and size between endemic and non-endemic butterflies. The specimens were not collected for biodiversity monitoring purposes and so are likely to have collection biases reflecting the interests and opportunities available to the collectors (Meineke & Daru, 2021). Individual collectors may have focussed more on some species than others (e.g., more colourful and larger species) or may have prioritised sites at low elevations for convenience and accessibility and/or on sites with known high species richness. Nonetheless, we find considerable variation in the elevations of where our study species were recorded (overall range, 0–1829 m.a.s.l), but that specimen collection primarily occurred in the lowlands (67.2% of records were from sites <500 m a.s.l) and thus limit the power to detect elevation differences between endemic and non-endemic species. However, the patterns of collection of endemic and non-endemic species were similar (Table S3), although collecting biases obscure a detailed understanding of the full extent of species ranges. Contrary to our initial expectations, we find that endemic species occur at low elevations on Sulawesi, overlapping in elevational range with many non-endemic species

(Figure 2b), as found in other species accounts (e.g., Nakae, 2021). We note that two endemic species did not occur at sea level (Figure 2b), and visual inspection of Figure 2b shows that endemic species tend to have distributions that span a wider range of elevations, including higher elevations. More studies are needed to examine whether upper elevation limits differ between endemic and non-endemic species, particularly in tropical regions. In addition, our study focussed on Papilionidae, because of the large number of specimens in museum collections from this family, which historically has been collected in great abundance due to the large and colourful appearance of species in this family. However, findings from this family may not represent other taxonomic groups, for example taxa with smaller bodies, different hostplant associations, phylogenies and/or life history traits, and so more studies are needed on a wider range of taxa.

These museum specimen data are a major part of known records for these species, and most of the specimens analysed in this study (89.6%) were collected before 1950, representing an invaluable resource of historical morphology data. Natural history collections are an invaluable and diverse vault of historic biodiversity data (Meineke et al., 2018; Pyke & Ehrlich, 2010), and these historic data remain underutilised in many fields, including conservation (Andrew et al., 2018; Greve et al., 2016; Kharouba et al., 2018). Combining with modern biodiversity data, these historic data can be used to investigate temporal changes in species distributions and morphology over time, as well as reveal patterns of community change over the past two centuries, a period of rapid acceleration in climate change and land-use change. These long-term biodiversity data are vital for informing conservation action amidst continued anthropogenic pressures (Magurran et al., 2010).

CONCLUSIONS

We found from specimens held in museum collections that endemic butterflies do not disproportionately occupy higher elevations on Sulawesi, implying that they are not any more vulnerable to climate-driven mountaintop extirpations than non-endemic species. Lowland species have more opportunities to shift their range upslope in response to climate change, and so high elevation sites can provide climate refuges, even though these species may still be threatened from continued loss of lowland forest. Inter-specific variation in size and melanism among Sulawesi butterfly species does not appear to be primarily driven by temperature, and we find no evidence for evolutionary adaptation to climate change in endemic species, based on morphological traits. We found that endemic species are less melanised than non-endemics, and that melanism is disproportionately concentrated towards the outer edges of the wings, which may indicate a role in enhancing wing structural strength, pathogen protection or sexual signalling. Further work should investigate the thermoregulatory consequences of paler wings in endemics and thermoregulation in tropical species more widely, and how climate warming will affect tropical species, especially geographically restricted species of conservation importance. Our work highlights the importance of scientific

museum collections for providing data to conservation and ecological studies.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article. The NHMUK specimen data are available in the NHMUK Data Portal, https://data.nhm.ac.uk/.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

Table S1. Data on the number of specimens of swallowtail butterfly species (Papilionidae) from Sulawesi (Indonesia) in the Natural History Museum (NHMUK, United Kingdom) and the Zoologische Staatssammlung München (ZSM, Germany) Collections. Of the 1044 male specimens found, two specimens were damaged.

Table S2. Data from repeat measurements to examine the robustness of the measurements. One specimen was selected from five species and each individual was repositioned and imaged ten times and measured using ImageJ.

Table S3. Contingency table showing the number of endemic and non-endemic specimens collected above and below the median elevation represented by all specimens analysed (150 m).

Figure S1. Map of Sulawesi (Indonesia), showing elevation (graded shading, based on 90-m resolution de Ferranti STRM DEM) and natural forest cover in 1950 (green, based on Hannibal, 1950), when 90% of the specimens analysed in our study were collected and earliest vegetation cover map available. The points show the specimen locations, the arrows highlight the major collecting hotspots and the inset map shows location of Sulawesi within southeast Asia. Map created using QGIS 3.14 (QGIS.org, 2020).

Figure S2. Set-up of (a) imaging equipment and (b) pre-formatted specimen tray. In (b), the thin yellow lines denote the forewing length measurement.

Figure S3. Variation in melanism (% black of dorsal body) across different RGB colour thresholds from a threshold of 0 (i.e., all white) to 1 (i.e., all black) for 29 Papilionidae species from Sulawesi (Indonesia).

Figure S4. Sensitivity of estimate of melanism for different RGB colour thresholds for 29 species from Sulawesi (Indonesia). The y-axis ('mean difference in melanism proportion between Focal $+ 0.1$ and Focal $- 0.1$') shows the arithmetic mean of the difference in melanism proportion between a given threshold (e.g., 0.6) and the melanism value at 0.1 above (i.e., 0.7) and below (i.e., 0.5) the threshold across specimens in each species.

Figure S5. The temporal distribution of endemic (purple shading) and non-endemic (yellow shading) swallowtail butterfly specimens. The 'Pre-1940' category represents specimens for which exact collection date is not known, but determined to be collected before 1940 from the accession date to the museum collection. The total number of specimens (a) and species (b) collected each decade are shown.

Figure S6. Species elevational ranges estimated in our study from specimens held at NHMUK and ZSM (x axis; 'Collections') plotted against species elevational ranges estimated from Global Biodiversity Information Facility (GBIF) occurrence records (y axis; 'GBIF Data'). Plot (a) shows species elevational ranges estimated only using GBIF records with elevation data (i.e., 271 records across 23 species) and plot (b) shows species elevational ranges estimated using all GBIF records (i.e., 3253 records across 28 species). Data points are median elevation values per species (endemic $=$ purple, non-endemic $=$ yellow), with the error bars representing the upper and lower quantiles.

Data S2. Supporting Information.

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