Low cold tolerance of the invasive lizard *Eutropis multifasciata* constrains its potential elevation distribution in Taiwan

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ABSTRACT

1. The invasive many-lined sun skink, *Eutropis multifasciata*, is established in much of southern Taiwan and is spreading northward. We investigated whether winter temperatures constrain further dispersion of this skink by comparing its cold tolerance to the spatial distribution of winter temperatures in Taiwan.
2. We measured the 28-day survival rate of this species at 4 constant temperatures ($10 - 16$ °C in 2 °C increments) and its critical thermal minimum (CTmin), i.e., the body temperature at which the righting reflex is lost during the cooling process. For comparison with the spatial distribution of temperatures over Taiwan, we used the biophysical model Niche Mapper™ in order to simulate the soil temperatures, where lizards are inactive in the winter, during the coldest month of the year, January, under three climatic scenarios (average temperature, average $- 3$ °C, average $+ 3$ °C).
3. Our results indicate that this species has low tolerance to cold. Combining cold tolerance data with soil temperature data suggests that its upper elevation limit could range from 1000 m to 1500 m, above which the weather is lethal and precludes overwintering. The locations of sightings of *E. multifasciata* are consistent with this prediction, with no known locations above 500 m elevation.
4. This study highlights that the winter climate is a major factor in determining population establishment and hence in limiting this species' range. Future studies would benefit from accounting for low winter temperatures and their potential influence on range limits of invasive species.

1. Introduction

Human activities have a variety of negative influences on biodiversity, including facilitation of a species' expansion from its native area (Dukes and Mooney, 1999). Invasive species affect indigenous communities by competing for resources and changing species interactions (Chao and Lin, 2017; Losos et al., 1993; Rodda, 2003). Invasive alien reptiles pose a threat for the conservation of biological diversity (Kraus, 2009; Wilcove et al., 1998). To inform actions for management, it is necessary to understand the biological characteristics of the invasive species, how well they adapt to their novel environments (Stahl et al., 2016; Walther et al., 2009), what limits their range, and what is their potential distribution boundary.

Climatic conditions play a fundamental role in determining distributional ranges of species (Andrewartha and Birch, 1954; Krebs, 2009). For ectotherms, temperature crucially affects range limits (Angilletta, 2009; Spellerberg, 1972) because many physiological and behavioral functions are temperature dependent (Bennett, 1980; Huey

and Stevenson, 1979). When a species of reptiles spreads to a novel environment, it must not only perform well in the active season but also endure adverse conditions during the inactive season. For reptiles originating in the tropics, cold events during winter are reported to be a critical factor affecting survival (Mazzotti et al., 2011), suggesting that winter conditions could limit their spread in new environments. Cold tolerance of reptiles has been shown to closely correlate to their latitudinal and elevational ranges (Campbell-Staton et al., 2018; Huang et al., 2006; Spellerberg, 1972).

The overwintering outcome of an individual, such as its survival, depends on the animal's cold tolerance and the microclimatic conditions experienced (Mazzotti et al., 2011). By linking cold tolerance to winter temperatures in the field, we can estimate the maximum range limit of an invasive species over a geographic region as imposed by winter temperatures. Traditionally, cold tolerance is estimated by the critical thermal minimum (CTmin), which, for reptiles, is often measured as the body temperature (T_b) at which mobility (or righting reflex) is lost as its body cools (Cowles and Bogert, 1944; Spellerberg,
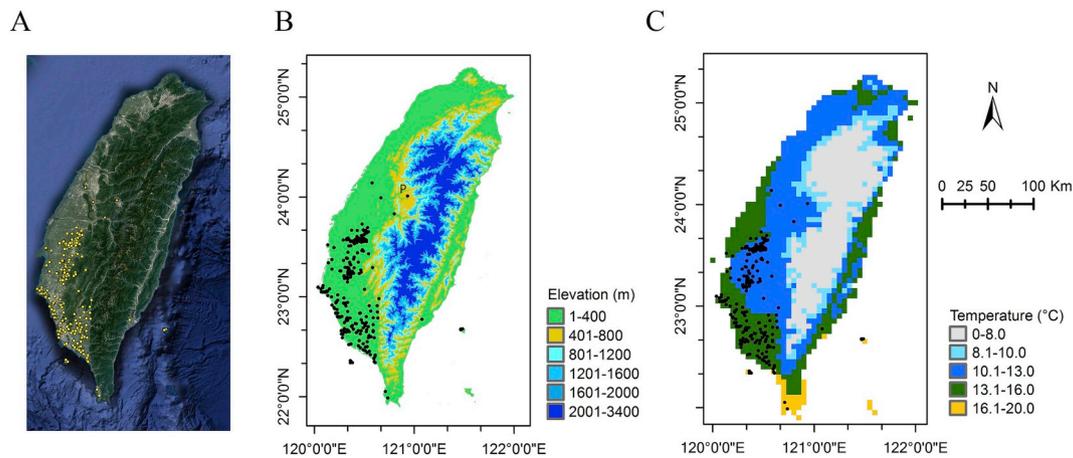

Fig. 1. Sighting locations of *E. multifasciata* on (A) an aerial photo (B) a digital elevation map and (C) January minimum air temperatures of Taiwan. Sightings are shown with yellow dot in (A) or black dots in (B) and (C) (n = 442). The Puli site (denoted by the letter P) is located at the elevation of 480 m. Temperature data: Average January minimum air temperature (data duration: 2005–2014, TCCIP, https://tccip.ncdr.nat.gov.tw/). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

1972). When an individual's T_b is below CTmin in the field, an individual loses the ability to move away from harmful conditions and may therefore die if the temperature continues dropping. The CTmin can be used to estimate the capacity of an invasive species to disperse in the winter. Another approach is to measure chronic impacts of cold, for example, by measuring the survival time of individuals exposed to a cold gradient (Huang and Tu, 2008).

The many-lined sun skink, *Eutropis multifasciata* (KUHL, 1820), is native to tropical Indonesia and southeastern Asia (Uetz and Hošek, 2016, http://www.reptile-database.org/.), but is an invasive species in Taiwan. This skink was first documented in southern Taiwan in 1992 (Ota et al., 1994), and since then has established stable populations in central and southern areas and is spreading northward (Fig. 1A and B, for details on sighting data, see Materials and Methods). *Eutropis multifasciata* has already negatively affected reptile diversity by directly preying on or competing with indigenous lizard species on Green Island (Chao and Lin, 2017). Currently, this skink is restricted to warm lowland areas in the plains, located in the southern and central regions of Taiwan. No sightings have been reported in the mountainous regions, which constitute about two-thirds of the total area of Taiwan (aerial photo, Fig. 1A). Information on the natural history and ecology of this species in its natural distribution is lacking. It was described to be inactive in the winter in the central highlands of Vietnam (Ngo et al., 2014) and southern China (Ji et al., 2006a). In Taiwan, this species was observed to be active throughout the year in southern areas (e.g., Yulin, Tainan, Kaohsiung, and Pintung County, Lin T. N. observations) and on Green Island (Chao and Lin, 2017), but the overwintering behavior is unclear. During the active season, this species prefers open habitats in agricultural and disturbed areas.

The aim of this study is to investigate the effects of winter temperatures on the distribution of *E. multifasciata* in Taiwan. We used an integrative methodology to approach this aim. First, we estimated the current distribution range from an occurrence dataset obtained from a citizen science project (Fig. 1A). As winter temperatures vary across areas of Taiwan (Fig. 1C), we assumed the distribution of *E. multifasciata* could be affected by its tolerance to cold. We measured the cold tolerance of two populations and then compared these results to the winter temperatures in Taiwan. The soil temperature at a depth of 20 cm, rather than air temperature, more closely reflects the actual temperature environment experienced by this surface-dwelling skink in the field in winter. We used the biophysical Niche Mapper™ model (McCullough and Porter, 1973; Porter and Mitchell, 2006) to simulate the spatial distribution of soil temperatures in the coldest month of the year, January, in Taiwan. In addition, in order to account for temperature variation among years, we

simulated the soil temperatures for 3 temperature scenarios: average years, warm years (average + 3 °C) and cold years (average − 3 °C), using the climate dataset from the most recent decade (2005–2014, Taiwan Climate Change Projection and Information Platform Project (TCCIP), https://tccip.ncdr.nat.gov.tw/, details see Materials and Methods). Finally, we used the cold tolerance data obtained from these populations as an example of how comparing soil temperature data with occurrence data provides an insight into the effects of winter on the skink and suggests its potential maximum distribution limits in Taiwan.

2. Materials and Methods

2.1. Study animal

The many-lined sun skink is a viviparous lizard (adult snout-vent length (SVL), less than 13 cm). In Taiwan, females produce 1–15 offspring per clutch and reproduce twice per year at most in the field (T. N. Lin personal observation). This species is well adapted to agricultural areas and human-disturbed regions. Its diet includes insects and other small invertebrates, as well as other small lizards.

2.2. Occurrence data

The occurrence data of *E. multifasciata* were obtained from two databases, the roadkill database of a citizen science project (https://roadkill.tw/en; Endemic Species Research Center, Taiwan) and the database from Taiwan Biodiversity Network (https://www.tbn.org.tw). The roadkill database comprises more than 118000 sightings of roadkilled wild animals in Taiwan. The sightings of reptile species span a wide range of elevations (from sea level to 3125 m elevation, Tables S1 and S2 in Supplementary Materials). Sightings from the Taiwan Biodiversity Network were collected from field surveys by Taiwan Endemic Species Research Institute.

We also assessed the quality of the roadkill database for estimating the elevational range of *E. multifasciata* by analyzing elevational patterns in the data for all lizard and snake species (34 lizard species, n = 2874 sightings; 55 snake species, n = 15113 sightings, duration: 2008–2017; Table S1 and Table S2 in Supplementary Materials). The sampling efforts could be affected by many factors such as spatial distribution of the roads, length and conditions of roads. Instead of attempting to standardize the sampling effort, we calculated the total number of the individual snakes and lizards in 7 elevational ranges (sea level − 3250 m in 500 m increments). Details of these sightings are listed in Table S3 and Table S4 (Supplementary Materials).

2.3. Measurement of cold tolerance

2.3.1. Collection and maintenance of animals

We collected adult lizards from Kaohsiung (22°37′43.6″N 120°16′10.5″E) and Yunlin (23°41′28.6″N 120°27′36.9″E) from August to October 2018. Lizards collected from Kaohsiung were used to measure CTmin (17 females and 19 males) and chronic cold tolerance (30 females, 26 males). None of the animals were used more than once in our experiments. Lizards collected from Yunlin (11 females and 21 males) were used to measure CTmin only. Lizards were housed in the laboratory individually in plastic boxes (length × width × height = 30 cm × 17 cm × 22 cm) containing dry grass and a piece of tile as a shelter. They were provided with water and food (crickets and mealworms dusted with vitamin powder) *ad libitum*. The temperature was set to 25 °C and the photoperiod was set to 13 h light: 11 h dark in August–September or to 12 h light:12 h dark during October–December. The experiments were conducted in November and December 2018.

2.3.2. Measurement of CTmin

The lizards were divided into three groups with similar sex ratios and similar body sizes (Kaohsiung: females: SVL = 10.45 ± 0.58 SD cm, n = 17; males: SVL = 10.87 ± 0.80 SD cm, n = 19; 5–6 females and 6–7 males for each group; Yulin: females: SVL = 9.60 ± 0.97 SD cm, n = 11; males: SVL = 10.06 ± 1.00 SD cm, n = 21; 3–4 females and 7 males for each group). These groups were then randomly assigned to 3 temperature groups (16, 20, or 25 °C). We gradually decreased their temperature (1 °C/day) from room temperature to their assigned acclimation temperatures using temperature-controlled chambers (Model 740, HiPoint Corp., Taiwan; MIR- 554, Panasonic Healthcare Co., Ltd., Osaka, Japan). The CTmin was measured after the individuals had acclimatized at their assigned temperature for 2 weeks.

Each individual's T_b was measured with a thermocouple probe (K-type, 0.1 mm in diameter) inserted 1 cm into its cloaca. The probe was secured to its tail with surgical tape and connected to a thermometer (CENTER-306, Center-309, Center Technology Corp., Taiwan). The test individual was placed in a plastic box (length × width × height = 30 cm × 20 cm × 18 cm), which was then immersed in a chamber (length × width × height = 39 cm × 31 cm × 10 cm) containing 0 °C ice in a 25 °C room. We decreased the lizard's T_b by 0.6–0.9 °C/min. Once T_b of the lizard fell below 16 °C, we examined the lizard's righting reflex every 30 s by turning the lizard upside down and stimulating its pelvic region with pencils. The CTmin was defined as the T_b at which the animal loses its righting reflex (Spellerberg, 1972). The T_b was considered to be its CTmin when the lizard did not right itself within 30 s.

2.3.3. Survival time in four cold treatments

The lizards were divided into four groups with similar sex ratios and similar body sizes (females: SVL = 98.6 ± 10.7 mm SD, n = 30; males: SVL = 97.6 ± 11.2 mm SD, n = 26; 7–8 females and 6–7 males in each group). These groups were randomly assigned to one of four treatments (10, 12, 14, or 16 °C). Animals were housed individually in plastic boxes (length × width × height = 30 cm × 17 cm × 22 cm) containing dry grass and tile shelters. The temperature of the lizards was gradually decreased (1 °C/day) from room temperature to their assigned temperature using temperature-controlled chambers (Model 740, HiPoint Corp., Taiwan; MIR- 554, Panasonic Healthcare Co., Ltd., Osaka, Japan). They were maintained in the temperature controlled chambers at their assigned temperature, with light: dark set to a 12 h:12 h photoperiod. They were provided with water and food (mealworms dusted with vitamin powder) *ad libitum*. The survival status was checked every day for 28 days.

2.4. Simulation of soil temperature

We simulated soil temperatures for the coldest month of the year, January, using a Niche Mapper™ model (Porter and Mitchell, 2006) with a spatial dataset (5 km × 5 km resolution, total 1324 grid cells). We chose the middle day of January (January 15th) to represent January in the simulation. As we suspect that *E. multifasciata* only uses natural fissures as overwintering shelters that allow them to move underground and avoid the cold temperatures of the ground surface, we simulated soil temperature at 20 cm underground, which is a conservative estimate of the depth of a typical fissure. Niche Mapper™ requires locality (longitude, latitude), topographic attributes (aspect, slope, elevation), substrate properties (substrate thermal conductivity, substrate density, substrate specific heat capacity, substrate IR emissivity) and climatic data (daily minimum and maximum values of air temperatures, wind speeds, cloud cover and relative humidities) for each grid cell to simulate soil temperatures. For detailed parameters of Niche Mapper™ and values see Table S5 in Supplementary Materials. For these simulations we assumed flat ground for each grid cell. The elevation of each grid cell comes from TCCIP. We assumed that each grid cell contains both areas covered with sparse vegetation, and areas with heavy vegetation, available for lizards to choose from for overwintering. The soil temperatures for sparse and heavy vegetation were simulated by setting the values of a parameter, shade level, to low shade level (LS, shade level = 20%) or high shade level (HS, shade level = 80%) respectively, corresponding to 20% and 80% of solar radiation blocked by the forest cover. The wind speed was set to one of two levels (low: 0.1–2.0 m s^{-1}, high: 2.1–4.0 m s^{-1}), and cloud cover was set to be 54–66%, approximately averaged values estimated from the data from weather stations (Tables S6 and S7 in Supplementary Materials). In addition, we used values of daily maximum and minimum air temperatures estimated from 15 agricultural meteorological stations to simulate soil temperature at 20 cm deep using Niche Mapper™ (data duration: January 2010; Table S8 and Fig. S1, Supplementary Materials, Taiwan Central Weather Bureau; https://www.cwb. gov.tw/V7e/climate/agri/agri_10day_all.php_). We then compared the simulated soil temperature with the mean soil temperature recorded at each station. The information specific to each stations is the locations (longitude, latitude), mean maximum air temperatures and mean minimum air temperatures (Table S8, Supplementary Materials); two other parameters were set to the same value for all stations: wind speed of 0.1–2.0 ms^{-1} and shade level of 1%. Values for all other parameters are listed in Table S5 (Supplementary Materials).

In addition, in order to account for temperature variation among years, we simulated the soil temperatures for 3 temperature scenarios: average years, warm years (average + 3 °C) and cold years (average − 3 °C). For simulating each grid cell, the model uses the values of the daily minimum and maximum air temperature. For the average-year scenario, we set those values based on the mean daily minimum and maximum air temperatures collected from the TCCIP for the most recent decade (2005–2014). The 3 °C difference used in the warm and cold year scenarios corresponds to an empirical estimate of the maximum inter-annual deviation from January's average temperature. This estimate was calculated based on the data from five weather stations (lowland stations: Taipei, Taichung, and Kaohsiung; highland stations: Alishan, Yushan, recording duration: 2009–2018, Taiwan Central Weather Bureau). The maximum difference from the average for the cold year was 3.1 °C at the Taipei station in 2011 and 2.1 °C for the warm year at both Taichung and Kaohsiung stations in 2017. Values for other parameters (e.g., wind speed, humidity, etc.) used in simulation are listed in Table S5 (Supplementary Materials).

2.5. Data analysis

The data were tested for normality with the Shapiro-Wilk test, and homogeneity of variance was examined with the Levene test. We used

two-way analysis of variance (two-way ANOVA) to test the effects of acclimation temperatures (16, 20, 25 °C) and populations (Kaohsiung and Yunlin) on CTmin. The Tukey test was used for post-hoc comparisons. We used the Kaplan–Meier method to analyze whether survival time differed among the four temperature treatments. All tests were performed using the JMP statistical software (SAS institute, 1998).

3. Results

3.1. Occurrence data

A total of 303 sightings were collected from the roadkill database (sighting period: 2008–2018) and 139 sightings were collected from the Taiwan Biodiversity Network database (sighting period: 1999–2018). The vast majority of these sightings (n = 433) were located in the lowland agricultural area south of the Zhuoshui river in western Taiwan (Fig. 1A), except for the four northernmost sightings (Dadu, Caotun, Jiji and Puli). Among these four sites, only the Puli site (480 m elevation) has a confirmed established population (Fig. 1B). There were only five sightings in east Taiwan, one near Taitung City, and four on Green Island. All sightings of *E. multifasciata* were located below 500 m.

Results of analysis of elevational patterns of all lizard and snake species from the roadkill database is shown in Supplementary Materials (Table S1, Table S2, and Fig. S2). In general, the number of observations of lizards and snakes decreased with increasing elevation, with an obvious decline from the range of sea level −500 m (n = 13966 individuals) to the range of 501 m–1000 m (n = 2629 individuals). Nevertheless, a large number of observations (lizards: n = 399; snakes: n = 3622; total 4021 sightings), approximately 22.4% of total roadkill samples (n = 17987), were collected in areas above 501 m elevation. These results suggest that the elevation range estimated for *E. multifasciata* (below 500 m elevation) is very unlikely to have resulted from sampling bias.

3.2. Cold tolerance

3.2.1. CTmin

The CTmin was significantly affected by population effect and the interactions of acclimation temperature and populations (population: $F_{1, 62} = 9.22$, p = 0.003; acclimation temperature: $F_{2, 62} = 1.65$, p = 0.19; interaction: $F_{2, 62} = 3.80$, p = 0.02). The pairwise comparisons revealed that most groups were not significantly different in CTmin, except for the two populations compared at 25 °C acclimation (Kaohsiung = 13.3 ± 0.6 °C, Yulin = 11.6 ± 1.1 °C SD, Table 1). The mean lowest CTmin was 12.1 ± 1.2 °C SD and 11.6 ± 1.1 °C SD in the Kaohsiung and Yunlin populations, respectively.

3.2.2. Survival time at different temperatures

The survival time of lizards significantly dropped with decreasing temperatures (Log-rank Chi-square = 92.73, df = 3, n = 56, p < 0.0001, Fig. 2). All lizards died over the 28-day experimental period in the three low temperature treatments (10, 12 and 14 °C). The

Table 1
Critical thermal minimum (CTmin, mean ± SD) of *E. multifasciata* after two weeks acclimation at three different temperatures.

Location	Acclimation temperature	Sample size	CTmin[a]
Kaohsiung	16 °C	11	12.2 ± 1.3 °C[A,B]
Kaohsiung	20 °C	12	12.1 ± 1.2 °C[A,B]
Kaohsiung	25 °C	13	13.3 ± 0.6 °C[A]
Yunlin	16 °C	10	11.7 ± 0.9 °C[B]
Yunlin	20 °C	11	12.0 ± 0.7 °C[B]
Yunlin	25 °C	11	11.6 ± 1.1 °C[B]

[a] Different letters indicate a significant difference between two groups (2-way ANOVA, Tukey Post-hoc test).
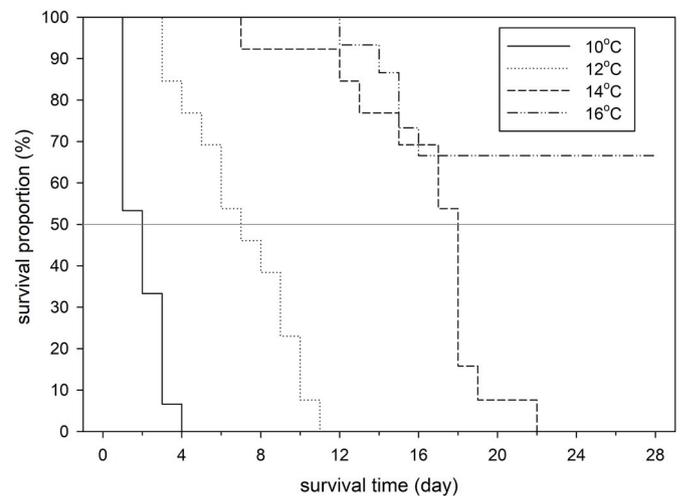

Fig. 2. Survival curves of *E. multifasciata* at four constant temperatures. The survival time significantly decreases with decreasing temperatures. The crossing of the horizontal grey line with each curve indicates the time at which 50% of individuals have died. n = 13–15 for each group.

median survival time was only 3 days when exposed to 10 °C, and all lizards died in 4 days (n = 15). In 12 °C and 14 °C treatments, the median survival time was 8 days (n = 13) and 19 days (n = 13), respectively. At 16 °C, 66% of the lizards survived to the end of the experiment.

3.3. Simulated soil temperature and suggested overwintering outcomes

The comparison of soil temperature data with simulated soil temperature of 15 agricultural stations is provided in Table S8 (Supplementary Materials). The simulated soil temperatures of these agricultural stations were slightly higher than measured soil temperatures (1.03 ± 1.07 °C SD, n = 15), and the difference between measured and predicted soil temperatures remained smaller than 2 °C for most of these stations (i.e. for 12 out of the 15 stations, Table S8 in Supplementary Materials).

Fig. 3 shows the simulated soil temperatures for high wind speed (2.1–4.0 ms^{-1}). As the results for low wind speed (0.1–2.0 ms^{-1}) were similar (Fig. S3, Supplemental Materials), only results of Fig. 3 are described in the main body of this paper. The simulations showed that soil temperature decreases with increasing elevation and latitude in general (Fig. 3; Fig. 1B). Most sightings were documented in the areas where soil temperature was estimated to be greater than 14.0 °C (Fig. 3A; Fig. S3A in Supplementary Materials) in average years, 12.0 °C in cold years (Fig. 3B; Fig. S3B in Supplementary Materials), and 16.0 °C in warm years (Fig. 3C, Fig. S3C in Supplementary Materials), except for the Puli and Jiji sites (denoted P and J, respectively, in Fig. 3). These two sites had cooler soil temperatures than the other sites.

In order to evaluate the potential elevation limit of this species, we compared the simulated soil temperatures (for high wind speed condition) found at three elevational ranges (sea level −999 m, 1000–1499 m, 1500–3400 m) with the two critical temperatures, 10 °C and 12 °C, measured in this study (Table 2). The lizards exposed to the 10.0 °C treatment died after a short time (Fig. 2), and 12.0 °C was a critical temperature for both CTmin (Table 1) and survival time (Fig. 2). CTmin may be important in determining survival in the field, for example, because occasional sharp drops in temperature may lead to death if lizards are not able to find underground shelter quickly. Only a small proportion of areas located below 1000 m had soil temperatures lower than 12 °C (Table 2). In the 1000 m −1499 m range, soil temperature was below 12.0 °C in most grid cells (65.8% in LS and 76.0% in HS), and below 10.0 °C in a few grid cells (26.7% in LS, 37.7% in HS). In the 1500 m −3400 m elevation range, most cells had a soil temperature

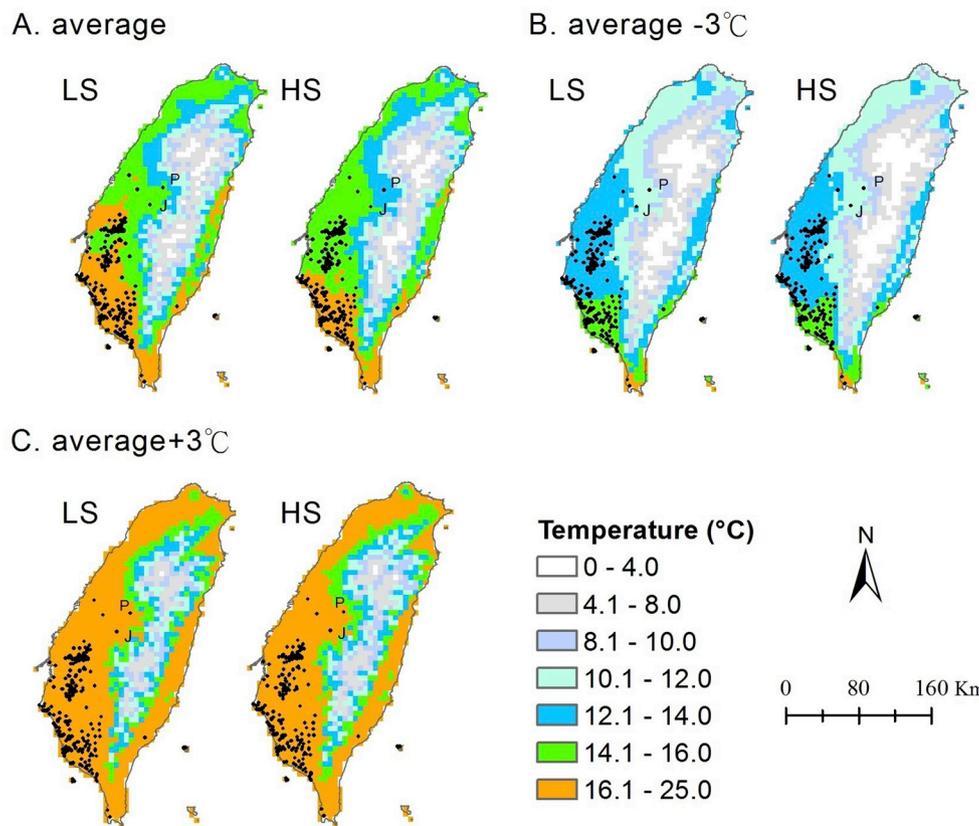## A. average

B. average -3°C

C. average+3°C

Fig. 3. Average soil temperatures simulated for January in the (A) average (B) average +3 °C and (C) average -3 °C temperature scenarios. LS: 20% shade level; HS: 80% shade level. Sightings are shown with black dots (n = 442). Notation: P: Puli site, J: Jiji site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

below 10.0 °C (89.1% in LS, 97.1% in HS).

The successful establishment of a population at a given location requires routinely suitable winter climate conditions, enabling individuals to survive. We used cold tolerance data obtained in this study and the simulated soil temperatures in the 3 climate scenarios to estimate the survival outcome of *E. multifasciata* in each grid cell. We followed the approach used by Di Santo et al. (2018) to classify the area of Taiwan into four overwintering zones, i.e., lethal, transient, transition, and survival zones. The lethal zone is defined as the area where winter soil temperatures are routinely lower than the lethal temperature (10 °C) (Fig. 2), suggesting that *E. multifasciata* could not become established in this area. The transient zone is defined as the area where soil temperature is between 10–12 °C for the average year scenario but is < 10 °C in the cold year scenario, suggesting that individuals could potentially survive under average winter temperature conditions but not in cold years. The transition zone is defined as the region where soil temperatures are higher than 12 °C in the average year scenario but are between 10–12 °C in the cold winter scenario. The winter temperatures in the transition zone may threaten survival during cold years because mobility could be lost. Lastly, the survival zone is defined as the region where the winter temperature is higher than 12 °C even in cold years, and is routinely higher than 14 °C, suggesting that winter survival is largely unaffected by temperature.

Fig. 4 shows the four suggested overwintering zones for *E. multifasciata* with simulated soil temperatures for high wind speed. As the results for low wind speed were similar (Fig. S4, Supplemental Materials), only results in Fig. 4 are described in the main body of this paper. The lethal zone is located in mountain areas higher than 1500 m, and comprises about 21.7% (LS condition) to 25.2% (HS condition) of the total area. The transient zone is below, and adjacent to, the lethal zone, and comprises about 16.3% of the total area in LS condition and 17.3% in HS condition. Below the transient zone are the transition and survival zones, which are located in lowland areas (1–600 m elevation range), with the transition zone located north of the survival zone (Fig. 4A and B). The transition zone comprises 22.1% of the total area in LS condition and 23.0% of the total area in HS condition. The survival zone comprises 39.9% and 34.6% of the total area in LS condition and HS condition, respectively.

3.4. Sensitivity test

The details of the sensitivity test are described in Supplementary Materials (Figs. S5, S6, S7). The model is sensitive to the settings of certain parameters such as wind speed and cloud cover. Changing the values of these parameters can lead to slight variations in the simulated range limit. Stronger wind speed and greater cloud cover values caused

Table 2
The percentage of cells for which the simulated soil temperature was below the critical temperature in three elevation ranges in the average climate scenario.

elevation range	sea level − 999 m		1000 m − 1499 m		1500 m − 3400 m	
total grid cells (n)	n = 905		n = 146		n = 273	
soil temperature[a]	< 12.0 °C	< 10.0 °C	< 12.0 °C	< 10.0 °C	< 12.0 °C	< 10.0 °C
LS condition	5.4%	0.1%	65.8%	26.7%	100%	89.1%
HS condition	7.3%	0.7%	76.0%	37.7%	100%	97.1%

[a] In reference to CTmin = 12.0 °C and critical temperature to rapid death = 10 °C; LS; shade level = 20%, HS: shade level = 80%, Setting of wind speed: 2.1–4.0 ms^{-1}.
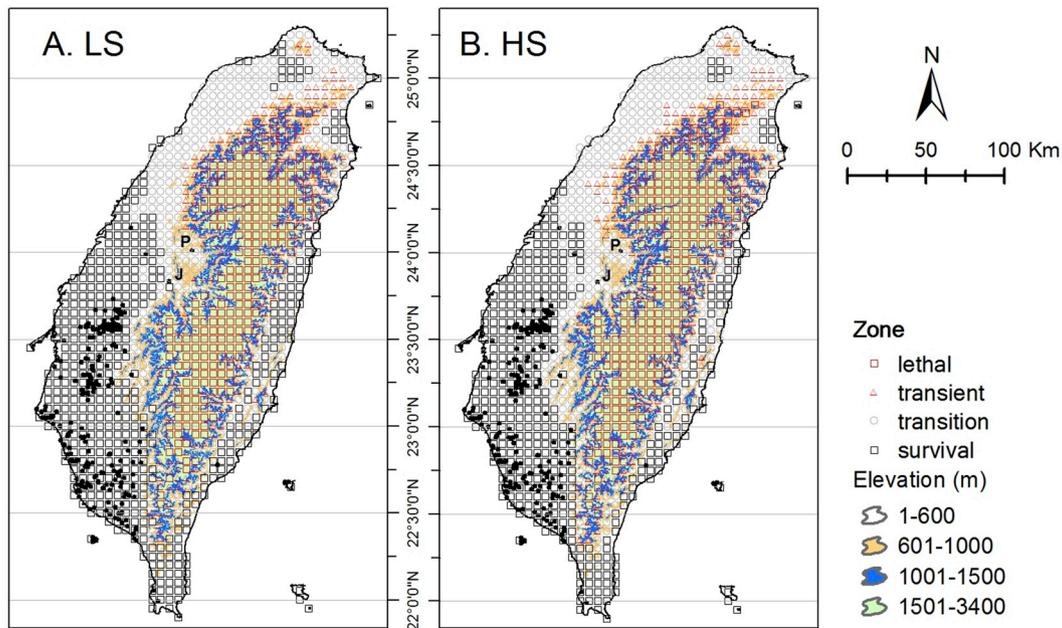
Fig. 4. Four suggested zones with differing potential overwintering outcomes for *E. multifasciata* in (A) LS and (B) HS conditions. LS: 20% shade level; HS: 80% shade level. Sightings are shown with black dots (n = 442). Notation: P: Puli site; J: Jiji site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

colder simulated soil temperatures (Fig. S5, Fig. S6 in Supplemental Materials), and therefore our results overestimated the soil temperaures for windy location such as open habitats in the mountains.

4. Discussion

An invasive species' spread is determined both by ecological attributes of its environment and by physiological attributes of the organism. This study investigates the impact of winter temperature on survival of the invasive species *E. multifasciata* and its distribution range in Taiwan. We measured its cold tolerance using animals obtained mainly from one population and estimated two critical temperatures to compare with simulated soil temperatures. Our results show that this skink has low survival tolerance to cold (Table 1, Fig. 2), which precludes its establishment in regions above 1500 m (Table 2). We further defined four zones with different winter outcomes in terms of individuals' survival and potential to maintain stable populations (Fig. 4). The occurrence data support the areas predicted in survival zone in general (Fig. 4). Accordingly, the geographic range of this species can be critically affected by winter climate and especially the temporal variation in winter temperature (Fig. 3).

4.1. Ecological implications of cold tolerance: distribution and population persistence

The cold tolerance data provide a solid physiological foundation to understand the overwintering outcomes in *E. multifasciata* and its influence on the potential distributional range of this species. The significant effect of acclimation temperature on CTmin was observed only in the Kaohsiung population, not in the Yunlin population (Table 1). The observation of a significant acclimation effect on CTmin is consistent with observations in many other reptiles (Campbell-Staton et al., 2018; Huang and Tu, 2008; Kour and Hutchison, 1970). A study of the invasive amphibian cane toad (*Rhinella marina*) also suggested that its rapid acclimation to colder ambient temperatures reduced its CTmin and facilitated its spread to mountain areas in Australia (McCann et al., 2014). A report of increased cold tolerances in the introduced population of an lizard (*Anolis cristatellus*) as compared to the population at the

original distribution may reflect acclimation or genetic changes after introduction (Kolbe et al., 2012).

The outcome of combining two critical temperatures with the simulated spatial soil temperatures indicates that *E. multifasciata* could be constrained to lowland areas (Fig. 4). By using two-cutoff points (10 °C and 12 °C) to evaluate the adverse impact of the thermal environment in each grid cell, our results indicate that in most regions in the middle elevation range (1000 m–1499 m elevation), the temperature could be harmful, and even lethal, to this species (Table 2). Accordingly, *E. multifasciata* is unlikely to survive winter in areas higher than 1500 m. While cold tolerance data could be used to estimate the lethal zone, it does not imply that areas outside the lethal zone are safe for winter survival. Mild cold (e.g., > 12.0 °C) could cause chronic detrimental effects on the lizards (Fig. 2), which we did not consider in defining the four overwintering zones of this study (Fig. 4).

The cold tolerance data can also be used to evaluate the effect of rare extreme cold events on *E. multifasciata*. Most simulation research, including our study, used air temperature databases that were already averaged for simulation, and hence could miss the critical impact of rare cold events on overwintering outcomes. However, one of the advantages of using a biophysical model is that it contains parameters that can account for extreme rare events (minimum and maximum daily air temperatures) to simulate soil temperatures. With original temperature recordings for rare cold events, we could evaluate their impact on this species. For example, on Jan 25, 2016, a cold snap caused the temperature to drop to 4.4 °C in central parts of Taiwan (24°08′50″N, 120°40′33″E, Taichung station, Taiwan Central Weather Bureau), which is near the northern limit of *E. multifasciata*'s distribution (Fig. 1B). The simulation of an extreme cold event predicts lethal soil temperatures (range: 6.5–11.5 °C, mean = 8.8 °C, LS condition), which could pose a strong impediment to the persistence of local populations. In this region, the minimum temperature in January was lower than 6.0 °C in 5 different years during the past 19 years (1990–2018, Fig. S8 in Supplementary Materials). Other empirical studies also report that winter cold snaps caused population declines and massive mortality for an invasive reptile, the Burmese python (Avery et al., 2010; Mazzotti et al., 2011).

While cold tolerance data proved useful for investigating the

potential range limit of *E. multifasciata*, it should be used with caution for other reptiles. By comparing the elevational distribution and CTmin of other lizards indigenous to Taiwan (Huang et al., 2006; Huang and Tu, 2008), we found that high-altitude lizards generally have a low CTmin (*Takydromus hsuehshanensis* = 2.0 °C, *Sphenomorphus taiwanensis* = 5.3 °C, distributional elevation > 2000 m). Yet, lowland reptilian species do not always have a high CTmin. For example, the lowland *S. incognitus* (distributional elevation < 500 m) has a high CTmin of 11.6 °C, but two other lowland *Takydromus* lizards have, in contrast, quite low CTmin values (*T. stejnegeri* = 5.0 °C, distributional elevation < 500 m, *T. formosanus* = 4.5 °C, distributional elevation < 1000 m). Therefore, for these two lowland *Takydromus* lizards, factors other than CTmin alone are likely to have a great influence in determining their elevational distribution.

4.2. Overwintering survival and distribution range limits

Winter temperature variation may determine local population persistence and hence the maximum range limit of *E. multifasciata* (Fig. 4). Compared to the average winter (Fig. 3A), the skink would have a greater area safe for overwintering during warm years (green and orange areas in Fig. 3C), and it may expand its distribution range; in contrast, it would have a smaller area safe for overwintering during a cold year (Fig. 3B), which could cause a shrinkage of its distribution or deter its invasion. In a species of butterfly, it has been found that a warm winter caused a range expansion by reducing winter mortality (Crozier, 2004).

The four suggested survival zones are supported with sighting data. The majority of sightings were located in the survival zone (Fig. 4). The two exceptions are the Puli and Jiji sites, which are located in the transition zone. Only the Puli site is confirmed to have a population established, whereas the Jiji site has only one sighting, from 2010. The soil temperature in these two sites is safe for this species in average years (12.1–16.0 °C, Fig. 3A), but is lethal during cold years (10.1–12.0 °C, Fig. 3B), and thus the persistence of the populations could be challenged in cold years. These results address the fact that climatic variation among years is as important to take into account as the average temperature value when predicting the potential range limit of invasive species.

There are some limitations of using cold tolerance data to evaluate the field overwintering success of *E. multifasciata*. First, our method has a limited ability to evaluate the harmful impact of prolonged cold. We defined the lethal T_b to be 10 °C, but exposure above 10 °C for a long period of time can also result in death (see Fig. 2). Therefore, our results may underestimate the lethal zone area. Also, it is not clear whether other behaviors, such as thermoregulation by basking, affect winter survival. If such behaviors are required for winter survival, we may underestimate the survival zone area. Second, the skinks' cold tolerance was measured in laboratory conditions, which are unlike conditions in the overwintering sites in the field, where the temperature can fluctuate during the day. Also, in order to estimate the range limit of this species, this study simulated the soil temperature on a 5 km^2 spatial scale. We simplified the impact of vegetation cover and other attributes of the microclimate (Huang et al., 2013, 2014) using reference values (Table S5. Model parameterization in Supplementary Materials). The soil temperature estimated by this study was thus not representative of all possible field temperature conditions experienced by the lizards. Further validation of the suggested distribution limits (Table 2) or survival zones (Fig. 4) can be achieved using the following approaches. Winter survival of this species can be monitored using a field transplant experiment (Chen et al., 2013; Krebs, 2009) in the sites of interest, e.g., outside the survival zone. However, this method cannot rule out the potential synergistic impact of climatic conditions (e.g., humidity, precipitation, etc) on individuals. Real field temperatures at the sites of interest can be measured to test winter survival in the laboratory under specified conditions. This method does not account for the potential

behavioral strategies (e.g., basking behavior) that could modify the temperature experienced. However, both of these research methods could be expensive and time consuming.

Clearly, the current elevational distribution of *E. multifasciata* (< 500 m elevation) can be explained by winter survival, but it can also be explained by the shorter activity seasons at higher elevation. For example, cold climates in mountain areas might prolong the gestation period of females and further constrain embryonic development and reproduction in viviparous lizards (Ji et al., 2006b). Gravid females of *E. multifasciata* from other areas (southern China) have been observed to have preferred body temperatures in the range of 27.6–30.8 °C (Ji et al., 2007), and gestation length has been prolonged under low temperature treatments in the laboratory environments (Ji et al., 2006a).

4.3. Future directions

The major contribution of this study is to demonstrate a methodology that combines cold tolerance data, sighting data and landscape soil simulations to estimate an invasive reptile's distribution limits. In addition to winter temperatures, the temperatures during the growth season are also critical for a species to establish and maintain populations. At present, little is known about the ecology and overwintering behavior of *E. multifasciata* in the field in Taiwan. Future research would benefit from field studies of its behavior and an examination of thermal sensitivity of functional traits such as the preferred body temperature range for activity, growth and reproduction. This information could be used in mechanistic models to simulate the potential distribution range of species (Kearney et al., 2008; Kearney and Porter, 2004). Potential trait changes during geographical spread and their relation to the new thermal environment is worth investigating (Kolbe et al., 2010, 2012) and could be achieved by comparing the thermal sensitivity of populations from the southern region to those at the distributional edge.

5. Conclusion

This study demonstrates that the low cold tolerance of *E. multifasciata* constrains its potential establishment throughout Taiwan. According to our analysis, this species is able to persist in most lowland areas in the southern Taiwan but not in north or in mountainous areas above 1000 m, which is in accordance with occurrence data. We were able to reach these conclusions by combining cold tolerance data with landscape soil temperatures and including temporal variation in winter temperature in the simulations. The method could be applicable to study the distribution limit of invasive ectotherms in general.

Author contribution

TEL: conceived the research, analyzed road-kill data, TYC: conducted the cold tolerance experiments; HLW and RR: prepared climatic data for simulation; SPH: attracted the grant, conceived the research, analyzed data, ran simulation and wrote the manuscript.

6. Declarations of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.03.015.

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