Over-ocean dispersal inferred from the saltwater tolerance of lizards from Taiwan

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Research

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Abstract

Background

For non-marine organisms, crossing the sea requires the challenges of dehydration and hypersalinity to be faced. Thus, the rate of water loss and saltwater tolerance determine the ability of species to disperse over sea and further influence species distribution. However, this association between physiology and ecology has rarely been investigated in terrestrial vertebrates. In this study, we examined the lizard species differently distributed across Taiwan and the adjacent islands to determine whether these physiological responses reflect the geographical distribution. We performed immersion experiments on individuals and eggs to test the rate of water loss and the saltwater tolerance in the six chosen species, that is, four native species (*Plestiodon elegans*, *Eutropis longicaudata*, *Diploderma swinhonis*, and *Hemidactylus frenatus*) and two introduced species (*E. multifasciata* and *Anolis sagrei*).

Results

For the individuals, the results showed that *P. elegans* had the highest rate of water loss and the lowest saltwater tolerance, whereas *E. longicaudata* and *E. multifasciata* showed the lowest rate of water loss and the highest saltwater tolerance. *D. swinhonis*, *H. frenatus*, and *A. sagrei* had medium measurements compared with the aforementioned species. On the other hand, for the eggs, only the rigid-shelled eggs of *H. frenatus* were not influenced by the water immersion and were incubated successfully after experimental treatment. In contrast, the parchment-shelled eggs of *E. longicaudata* and *D. swinhonis* lost or gained water dramatically in the experiments, and none of them hatched after immersion.

Conclusions

These interspecies differences in water loss and saltwater tolerance strongly suggest the heterogeneity of over-ocean dispersal ability among these lizards. Combined with the historical geology and the origin areas of each species, the inferences of the results largely explain the current distribution of these lizards across Taiwan and the adjacent islands. Furthermore, this study also helps to elucidate the potential dispersal of two invasive species, *E. multifasciata* and *A. sagrei*, for conservation purposes.

Background

Over-water dispersal makes terrestrial organisms move from one land mass to another. The capability and probability of this dispersal ecologically relate to the distribution of the creatures across lands and islands and then further evolutionarily influence biogeography and biodiversity [1–3]. Molecular and fossil evidence indicates that organisms may only spread outward between lands by crossing the ocean, rather than using land bridges [2, 4, 5], denoting that over-water dispersal is an important strategy for organisms. In the Anthropocene, although human transportation has become a path to crossing oceans for many introduced species, for a species invading new areas artificially, they could still spread out to adjacent areas naturally [6]. For example, Norway rats, which invaded islands around the world by artificial boats, dispersed to adjacent islands by natural drifting or swimming [7, 8]. Therefore, over-water dispersal not only

is important for understanding the distribution of native species but also plays a substantial role in determining the range an invasive species can reach.

Extreme saline environments and crucial water loss are major physical challenges for terrestrial organisms when they are drifting on the ocean [9–11]. High-salinity environments are a stress for organisms. Different species have their own salinity tolerances [11–13]; this variation influences the survival differences among species in marine floating, leading to the variance in ability of oversea dispersal at a species level that further influences the distribution of the species [13, 14]. However, research about the association between this physiological ability and distribution has mainly focused on plants, freshwater animals, and arthropods [e.g. 10, 15, 16]. The dispersal of terrestrial vertebrates is rarely explored. In reptiles, some freshwater and terrestrial species appear in saltwater wetlands or coasts, even surviving there for a long time [17–19], demonstrating that reptiles can tolerate higher salinity environments. Nevertheless, previous studies about salinity tolerance have mostly focused on marine or estuary species [18, 20, 21]. In the few studies on freshwater and terrestrial species, the salinity treatments were usually orally fed or injective [12, 22], whereas treatment with saltwater contact, the most relevant situation faced by a drifting individual, has rarely been conducted in previous literature. Furthermore, none of these studies link salinity tolerance to species distributions. Consequently, this key ability for crossing oceans, the saltwater tolerance of direct water contact and how it relates to distribution, has not been fully elucidated.

Dehydration is the most crucial challenge for organisms drifting on the ocean and the vital factor of saltwater tolerance. In reptiles, water loss occurs primarily through skin in addition to the loss through respiration [23, 24]. The water loss of the skin is related to the adaption to the climate of the habitat environment [25–27], whereby species living in hot and dry habitats lose water slower than those living in cold and wet habitat [27, 28]. In addition, the lower rate of water loss indicates that the species can retain water for a long time, which could delay death from dehydration [29, 30]. Thus, species with lower rates of water loss are more likely to survive a drifting period of over-water dispersal than species that lose water fast. However, the relationship between water loss and survival rate in the context of ocean crossing in vertebrates has rarely been reported.

For most reptiles, there are two different ontogenetic stages to ocean crossing: the individual stage and the egg stage. Currently, ocean crossing at the individual stage has been reported abundantly through swimming, floating, or rafting [1, 31–33]. Compared with large species which can swim for a long distance, floating and rafting are the more likely ways for small reptiles to cross oceans [1, 34]. In geckos, dispersal by natural raft or artificial boats has been suggested in some small species [35, 36]. In *Anolis*, it can float for a short time on sea water [34] to disperse between islands in their native places [1, 4]. On the other hand, it is probable that over-water dispersal occurs at the egg stage [35, 37]. The saltwater tolerance and water loss of reptile eggs may be majorly determined by the type of eggshell. Reptilian eggs are mainly divided into two types: parchment-shelled eggs and rigid-shelled eggs [38]. The shell of the former is thinner and less calcareous than that of the latter, resulting in differences in flexibility and permeability of water exchange. Parchment-shelled eggs are highly sensitive to environmental humidity [39], whereas rigid-shelled eggs, which have a dense and hard shell, could limit the exchange of water and substance from the environment [40]. Therefore, rigid-shelled eggs should have higher tolerance to seawater. Some gecko

studies have shown that eggs can tolerate sea water well after immersion treatment [41, 42]. For parchment-shelled eggs, successful incubation after immersion of sea water has only been reported in *Anolis sagrei* [43].

Taiwan and the adjacent islands are currently blocked by the sea (a depth of approximately 70 metres) from the Asian continent. However, the main island of Taiwan and the small islands between Taiwan and China were all connected to the Asian mainland before 1.55 Ma and during the Last Glacial Maximum in 26.5–18 ka, during which the sea level fell 135 metres (Fig. 1G)[44–46]. Therefore, Taiwan and these western islands are continental islands. Terrestrial species could disperse between Taiwan and Asian mainland through the land bridge (Fig. 1G). In contrast, the eastern ocean of Taiwan is drastically deep (a depth of over 1,000 metres) due to the oceanic trench in the nearby eastern coast, preventing the islands east of Taiwan from connecting to any island and mainland historically (Fig. 1G). Thus, the eastern islands are oceanic islands, where the species arrive only by over-water dispersal. Combining these historical contexts of geographic connection across Taiwan and these islands, knowledge of the variation of salinity tolerance among species could demonstrate how the current species distributions were formed.

In this study, we aimed to examine the heterogeneity of saltwater tolerance of small lizard species across Taiwan and the adjacent islands to determine their potential ability of over-water dispersal and then inspect the current distributions of these species and their saltwater tolerance within the context of historical geology. Six small lizard species, specifically four native species and two introduced species, were chosen (Table 1) in this study. The four native species were *Plestiodon elegans*, existing throughout Taiwan, the western and northern islands, and south-eastern China (Fig. 1A); *Eutropis longicaudata*, distributed in southern Taiwan, the southern islands, and mainland Southeast Asia (Fig. 1B); *Diploderma swinhonis*, existing throughout Taiwan, the southern islands, and the Ryukyu Islands (Fig. 1C); and *Hemidactylus frenatus*, existing throughout Taiwan, all adjacent islands, and almost every island in East and Southeast Asia (Fig. 1D). The two introduced species were *Eutropis multifasciata* and *Anolis sagrei*. The former is native to the Philippines and was first discovered in southern Taiwan in 1992 [47]. This species has been found in Green Island since 2008 [48] and in Orchid Island since 2017 (Fig. 1E). *A. sagrei* from the West Indies was discovered in central Taiwan in 2000 [49] and eastern Taiwan in 2006 [50] but has not been discovered to date in adjacent islands (Fig. 1F). In this study, we performed experiments of seawater immersion on these six species and their eggs to simulate the condition of floating on the ocean. Specifically, we first examined the water loss and the survival rate/incubation rate of these small lizards and their eggs using the immersion experiments to evaluate the possibility of natural cross-ocean dispersal. Second, we inspected the association between these variations in saltwater tolerance and the distributions of these six species with the historical contexts of geographic connection across Taiwan and the adjacent islands. Finally, in addition, we assessed the dispersal risk of the two introduced species for conservation purposes.

Table 1

Distributions of six lizard species in Taiwan and adjacent islands. The distance is the closest distance to Taiwan.

	Taiwan	Penghu	Little Liuqiu Island	Guishan Island	Green Island	Orchid Island	Philippines
Distance from Taiwan	NA	47 km, west	13 km, southwest	11 km, northeast	44 km, southeast	63 km, southeast	161 km, southeast
<i>Plestiodon elegans</i>	O	O	O	O	X	X	X
<i>Eutropis longicaudata</i>	O	X	O	X	O	O	X
<i>Eutropis multifasciata</i>	O	X	O	X	O	O	O
<i>Diploderma swinhonis</i>	O	X	O	X	O	O	X
<i>Hemidactylus frenatus</i>	O	O	O	O	O	O	O
<i>Anolis sagrei</i>	O	X	X	X	X	X	X
The direction is the relative position to Taiwan. The character O indicates that this species exists in that location, and the character X indicates no distribution.							

Results

Rate of water loss of lizards

Among the six species, the *P. elegans* individuals had the highest rate of total water loss (RTWL) in the control (Fig. 2A), indicating that this species loses water fast in the air. In *E. longicaudata* and *E. multifasciata*, the RTWLs of the control were low, and there were no significant differences between the control and freshwater treatments (FW) (Fig. 2B and F). Meanwhile, in the other four species, the RTWLs between the control and FW showed significant differences that were lower in FWT. In FW, except for the negative value of *D. swinhonis* (male: -0.069 ± 0.087 , female: -0.032 ± 0.058 ; Fig. 2C), which indicated that *D. swinhonis* drank water, the RTWLs of the other five species were not significantly different (male: 0.021–0.073, female: 0.019–0.063). The RTWLs were not different between males and females in all treatments in all species. Because most of the individuals in the saltwater treatments (SW) died before the end of the treatment period, resulting in the scarcity of RTWL data in this group, we did not include them in the comparison in this study.

Survival rate of lizards

The survival rates (90–100%) to the end of the treatment period were not different between the control and FW in all species (Fig. 3). In contrast, the survival rates of SW decreased significantly compared with the control and FW in most of the species, except *E. longicaudata* and *E. multifasciata* (Fig. 3). When treated with SW, the survival rates of the four influenced species were extremely low, indicating their low tolerance to saltwater (Fig. 3A, C, D, and E). Meanwhile, the survival rates of the two *Eutropis* species were not significantly affected by saltwater immersion (Fig. 3B and F). Sex was not associated with the survival rate in all treatments in all species.

Table 2 shows the survival in detail. *P. elegans* survived worse even during the treatment period where 90% of individuals in SW died on day one (Table 2). In contrast, the survival rates of *E. longicaudata* and *E. multifasciata* remained higher than 70% at the end of the treatment. In *D. swinhonis*, more than 70% of SW individuals survived on the first day; however, most of them died on the second day, and only 1 out of 20 SW individuals survived to the end of the treatment. In *H. frenatus*, the survival rate of SW was higher than 60% on the first day and 40% on the second day, and 4 out of 20 individuals survived to the end. The SW survival rate of *A. sagrei* was higher than 70% on the first day, and it was also 40% on the second day. None of them survived to the end of the SW treatment.

Table 2
Accumulated survival rates in six lizard species in the saltwater treatment.

Species	Sex-stage	N	Survival rate (%)		
			Day1	Day2	Day3
<i>P. elegans</i>	male	10	0	0	0
	female	10	10	0	0
<i>E. longicaudata</i>	male	6	100	83.3	83.3
	female	10	100	100	90
<i>D. swinhonis</i>	male	10	90	10	10
	female	10	70	0	0
<i>H. frenatus</i>	male	10	60	40	30
	female	10	80	40	10
<i>E. multifasciata</i>	male	10	100	90	70
	female	11	100	100	90.9
<i>A. sagrei</i>	male	10	90	60	0
	female	10	70	40	0

Rate of water loss of eggs

In the eggs of *E. longicaudata* and *D. swinhonis*, the RTWLs in the control and FW were lower than zero (Fig. 4A and B), indicating that the eggs in these two treatments absorbed water during the experimental treatments. The significantly lower RTWL in FW denoted that they took in much more water than the control. Meanwhile, higher RTWLs in SW than in the control and FW were shown in both species that lay parchment-shelled eggs. (Fig. 4A and B), indicating that the eggs of these two species lost water severely during SW. In contrast, the RTWLs in *H. frenatus* had no significant difference among treatments and were not different from zero, indicating that none of the treatments influenced the water within the eggs, and almost no net water loss/absorption occurred in these rigid-shelled eggs (Fig. 4C).

Hatching rate of eggs

In *E. longicaudata* and *D. swinhonis*, the hatching rates were similar between the control and FW (Fig. 4D and E), but those in SW were significantly lower, indicating that saltwater immersion indeed decreased the incubation rate for these two species. In contrast, in *H. frenatus* the hatching rate in the control treatment (100%) was not different from that in FW (100%) and SW (100%; Fig. 4F), denoting that none of the treatments affected the hatchling rate of these rigid-shelled egg.

Discussion

This study is a rare report testing the saltwater tolerance of terrestrial vertebrates with immersion experiments. Compared with most previous studies which have focused on marine or estuary species [18, 20, 21, 51] or those which only used orally fed or injective salinity treatments in terrestrial vertebrates [12, 22, 52]; in this study, we investigated both the RTWL and survival rate with saltwater immersion of these terrestrial lizards to evaluate the possibility of cross-ocean dispersal.

Saltwater tolerance of individuals among species

Among the native species, our result demonstrated that the RTWL of *P. elegans* was the highest (Fig. 2A) and the survival rate of SW was the lowest (Fig. 3A and Table 2), suggesting that over-ocean dispersal is less likely for this species. In contrast, *E. longicaudata* tolerated SW notably well, showing low RTWL in the control (Fig. 2B) and a high survival rate in SW (over 80%; Fig. 3B and Table 2), indicating that this species is more able to spread by drifting on the ocean. The other two native species, *D. swinhonis* and *H. frenatus*, had moderate tolerance to SW (Fig. 2C and D, Fig. 3C and D, Table 2). Compared with *P. elegans*, they may have better ability to cross oceans if the floating period is shorter than three days (Table 2). In introduced species, the RTWL and saltwater tolerance of *E. multifasciata* were similar to the native *E. longicaudata* (Fig. 2F and 3F, Table 2), indicating that this invasive species had similar potential to disperse across oceans as the native *Eutropis* in Taiwan. In contrast, *A. sagrei* tolerated SW the worst; none of the individuals survived the experimental period (Table 2), suggesting that they could not drift on the ocean for a long time. For *D. swinhonis*, *H. frenatus*, and *A. sagrei*, most of them died in SW; they may survive if they could cross the ocean to land within two days by drifting, which means that crossing small straits, especially among island series or archipelagos, is still possible. *A. sagrei*, for example, has been observed

entering the sea and swimming away in its native habitat, and it could arrive to adjacent islands by floating [1]. Therefore, it is still possible that *A. sagrei* invades the adjacent islands from Taiwan by drifting.

The variance in survival rate in SW may be determined by the difference in RTWL among species. Although we did not have these data in SW because of the high mortality, the RTWL values in the control and the differences between the control and FW still provides a glimpse that the amount of water loss varied among species and likely occurs in SW. Mechanically, this variance in water loss may be attributed to the differences in species body size (slower water loss in bigger species) [53–55], the amount of lipid content under the skin (slower water loss in species with more lipids) [25, 26, 54], and the morphology of scales (slower water loss in species with larger and fewer scales) [56]. We do not know the lipid contents among these species, but we did find that the size of the scales is relatively larger in *E. longicaudata* and *E. multifasciata* than in other species (personal observation). Meanwhile, body size is not the likely factor in this study because it was not significant in the RTWL analysis. Evolutionarily, phylogeny [23, 53] and adaptation to the habitat climate (slower water loss in species living in hotter and drier habitats) [27, 28] may be associated with the variance in RTWL as well. *Plestiodon* originated in temperate northeast Asian [57]; thus, it is physiologically adapted to low temperatures, whereas the other species originated in tropical areas [58, 59] with high temperatures. This long-term effect might lead to higher rates of water loss in *P. elegans* than in the other species.

Saltwater tolerance of eggs among species

In the egg experiment, the eggs of *E. longicaudata* and *D. swinhonis* tolerated SW the worst, as shown in the high RTWLs and notably low hatchling rates in SW (Fig. 4A, B, D, E). In contrast, the low RTWL and the high hatchling rate in *H. frenatus* regardless of the treatment indicate that the eggs of this species tolerate SW well (Fig. 4C and F), supporting the high tolerance to sea water in gecko eggs in previous studies [41, 42].

The difference in SW tolerance of the eggs among these three species may have resulted from the types of egg shell; the eggs of *E. longicaudata* and *D. swinhonis* are parchment-shelled, whereas those of *H. frenatus* have a rigid shell. The RTWL results in this study are consistent with previous studies showing that water permeability is usually higher in the former than in the latter [39]. We further extend this prediction from eggs in the air to eggs in the water. This variance may largely determine the egg tolerance to SW and further lead to the heterogeneity of ocean-crossing ability. Here, we suggest that successful natural drifting at the egg stage is more likely in *H. frenatus* than in *E. longicaudata* and *D. swinhonis*. The high SW tolerance of the gecko eggs shown in this study might partially explain the wide distribution and low genetic divergence of the populations of this species across lands and islands in Southeast Asia [59].

Interpretation of current distribution and dispersal routes in the native species

The results of this study largely reflect the current distribution of each species with the context of dispersal history and the historical topography around Taiwan. The low saltwater tolerance of *P. elegans* in this study

explains why this species is only distributed on the western islands, Penghu and Little Liuqiu Island but not on Green Island and Orchid Island; arrival to the oceanic islands must be through over-water dispersal. *P. elegans* diverged with a related species that existed in Japan and the Ryukyu Islands approximately 4.23 Ma [57]. At that time, the Ryukyu Islands and Taiwan were still connected to the East Asian continent. The ancestral species may have spread southward from the Ryukyu Islands to Taiwan via the land (Fig. 5) and then spread to the western islands without crossing the ocean. *Plestiodon* species, belonging to the same clade as *P. elegans*, are almost exclusively distributed on continent and continental islands [57, 60], and this supports the inference of the *P. elegans* dispersal route, as well.

The high saltwater tolerance of *E. longicaudata* demonstrates that this species has the ability to drift on the ocean for long distances and arrive on the next piece of land, explaining why this species is distributed in the oceanic islands, Green Island and Orchid Island. *E. longicaudata* evolved in mainland Southeast Asia approximately 38 Ma [58], possibly arriving in Taiwan via the land bridge when Taiwan was still connected to the Asian continent or through over-water dispersal by taking the oceanic current to Taiwan. Currently, *E. longicaudata* does not occur in northern Taiwan because of the limitation of low winter temperatures. However, if climate warming continues, it may spread northward in Taiwan and possibly cross the ocean to the north islands near Taiwan.

D. swinhonis may survive on the ocean for only one day according to our results. Therefore, ocean crossing over a long distance is unlikely for this species. The wide range of distribution they currently have (Fig. 1C) might have resulted from two routes: dispersal through the land bridge and over-water dispersal to the islands where they could arrive within one day. Because the distances between Taiwan and the adjacent islands are small (Table 1), forces from climate events, such as the monsoon or typhoons, may shorten the dispersal time to these islands to within one day. Therefore, almost every adjacent island, including the oceanic Green Island and Orchid Island, could be reached from Taiwan by this species. Their western island distributions could be explained by both land bridge dispersal and over-water dispersal, whereas the eastern island distributions resulted exclusively from the route across the ocean.

A noteworthy conflict between the results and current distribution occurs at Guishan Island. Guishan Island emerged above the ocean approximately 7,000 years ago [61] and was never connected to Taiwan by a land bridge. *P. elegans*, which has very low tolerance to saltwater, occurs on this island, while *D. swinhonis*, which has moderate saltwater tolerance, does not. In addition, *Diploderma* species spreading through the Kuroshio current have occurred on the Ryukyu Islands [5] but not on Guishan Island, which is also on the route of Kuroshio. One explanation is that over-water dispersal is a rare event for both species while it only accidentally occurred in *P. elegans*. Alternatively, perhaps the microhabitat on this small island is suitable for *P. elegans* but not for *D. swinhonis* as these two species occupy different microhabitats; that is, the former appears in dense bush, but there are no tall trees for the arboreal *D. swinhonis* on Guishan Island. There is a similar situation in Penghu, which is located in the land bridge (Fig. 1G). *P. elegans* exists there, but *D. swinhonis*, which originated from China, does not [62]. The environment of Penghu may not be suitable for *D. swinhonis* to survive permanently because the trees are too few for this arboreal species.

H. frenatus has the widest distribution across islands, which is not in line with its moderate saltwater tolerance, but it is accordance with the excellent egg tolerance to sea water in this study. This ability may be very helpful for colonizing islands naturally and partially explain the tremendously wide distribution of this species. Geckos spreading out through areas of human activity is currently well known [36, 63, 64]. However, the literature usually only considers the individual stage. In this study, we showed the high potential to disperse via ocean crossing during the egg stage, matching the speculation of a previous study [35, 37] and suggesting that the egg stage should be contemplated carefully in over-water dispersal in the future.

Interpretation of current distribution and dispersal routes in the introduced species

In the introduced species, there are different spreading possibilities to adjacent islands according to our results and the introduced history. *E. multifasciata* has been introduced artificially, and it has spread outward from Kaohsiung in southern Taiwan since 1994 [47]. This species might reach Green Island and Orchid Island by human transportation. However, the high saltwater tolerance of *E. multifasciata* in our results showed that it may also be able to disperse by drifting to these islands from Taiwan or the Philippines by ocean currents. More evidence is needed for confirming how the species arrived on these islands. Today, this species has adapted well to the environment in Taiwan, and we have shown they have great ability to cross the ocean. Thus, colonizing other islands near Taiwan is highly possible for them in the future, even the northern islands if the climate warming continues.

A. sagrei are believed to have been introduced to Taiwan by human transportation, too [49]. The medium saltwater tolerance of *A. sagrei* in our results showed that it may drift and survive in one or two days, which is long enough to arrive to adjacent islands. Compared with *E. multifasciata*, *A. sagrei* is highly unlikely to disperse to other islands naturally because of the low saltwater tolerance and the current limited distribution in Taiwan. We suggest focusing on human activities to prevent the further invasion of this introduced species.

Conclusion

Our results showed the heterogeneity of saltwater tolerance among lizard species, in both the individual and egg stage, which may reflect the ability of over-ocean dispersal at an interspecies level. At the individual stage, *P. elegans* is less likely to cross oceans, whereas the two *Eutropis* species, *E. longicaudata* and *E. multifasciata*, are highly likely to survive on the ocean for days and successfully colonize new islands. For eggs, the rigid-shelled eggs of *H. frenatus* have great potential in over-water dispersal; in contrast, parchment-shelled eggs are less likely to survive after sea water immersion. Combined with the historical geology and the place of species origin, our results mainly interpret the distributions of these species across the islands around Taiwan and further propose the possible dispersal routes of these lizards. In addition, in this study, we help to elucidate the two invasive lizard species for conservation; *E. multifasciata* has the potential to disperse to any islands naturally, and it should be carefully monitored in

future years. For *A. sagrei*, we should focus on the islands near Taiwan to prevent natural over-water dispersal and the islands that have flourishing agricultural transportation.

Materials And Methods

Sample collection and husbandry

Six species, *P. elegans*, *E. longicaudata*, *E. multifasciata*, *D. swinhonis*, *H. frenatus*, and *A. sagrei*, were collected and kept individually in a plastic tank (36 cm × 17 cm × 20 cm) with water, food, and a natural light-dark cycle in a laboratory. *P. elegans* and *H. frenatus* were collected from the Pakua Mountain Range (23.989–24.024°N, 120.576–120.596°E) in Changhua. *D. swinhonis* was collected from Dadu Mountain (24.156°N, 120.559°E) in Taichung. *E. longicaudata* and *E. multifasciata* were collected from Sandimen (22.736–22.724°N, 120.638°E) and Nanhe (22.443–22.453°N, 120.621–120.623°E) in Pingtung. *A. sagrei* was captured from Qixingtang (24.019°N, 121.626°E) in Hualien. All captured individuals were kept in standard conditions for one week for acclimation before performing the lizard experiment. The pregnant *E. longicaudata* and *D. swinhonis* females were kept separately and checked frequently for eggs, which were collected while fresh. The *H. frenatus* eggs were collected from the wild because the pregnant females failed lay eggs in the laboratory. These eggs were used in the egg experiment. The pregnant females of viviparous *E. multifasciata* were also checked for hatchlings, which were used in the lizard experiment as well. Due to the high variance in body size among the captured individuals of *P. elegans* and the huge size difference between *E. multifasciata* adults and new born hatchlings, the small individuals of these two species were treated as juveniles in the lizard experiment. Only *P. elegans* and *E. multifasciata* juveniles were used in the analysis because the juveniles of the other species were difficult to collect. After the experiments, all of the lizards were released back to the places of capture. All of the collection, husbandry and subsequent treatment procedures followed the Wildlife Conservation Act of Taiwan; the animal use protocols were approved by the National Museum of Natural Science (license No. 1061701832).

Water immersion experiment

In the lizard experiment, individuals of each species were randomly separated into three groups, which received the saltwater (SW), freshwater (FW), or control treatments. Individuals with SW were placed in a 36 cm × 17 cm × 20 cm tank with 3.5% SW. The water surface slightly exceeded the abdomen of the lizard but did not exceed the mouth. In FW, individuals were treated as the setting in SW but replaced SW with FW. For the control treatment, individuals were placed into a tank without water. Each individual was weighed before the treatment and daily in the three continuous days of treatment. During the experiment, the faeces and shedding scales of each individual were collected and weighed as well. For the species with toepads, *H. frenatus* and *A. sagrei*, We used Vaseline on the wall of the tank to prevent the lizard from sticking to the wall. The temperature of this experiment was controlled at approximately 27 °C, and the humidity was controlled between 45–55%. In total, 91 *P. elegans*, 49 *E. longicaudata*, 62 *D. swinhonis*, 61 *H. frenatus*, 93 *E. multifasciata*, and 60 *A. sagrei* individuals were used in this experiment. There were at least ten individuals of each species in each treatment, except for male *E. longicaudata* (only six or seven) because they were difficult to collect.

In the egg experiment, only eggs of *E. longicaudata*, *D. swinhonis*, and *H. frenatus* were used in this study. Eggs which had been through at least half of the incubation days were used because the developmental time of the reptile eggs would influence the tolerance to environmental change [65]. The incubation periods were approximately 35 d in *E. longicaudata* and 50 d in *D. swinhonis*. The eggs of these two species were laid and collected in the laboratory and then kept in 27 °C and 45–55% for 17 and 25 d, respectively, before the egg experiment. The incubation period in *H. frenatus* was 56 d, but most of the individuals were collected from the wild without a specific laying date; they were used in the egg experiment one week later from the date of collection. However, the incubation days in all samples were from 14 to 35 d (24.7 ± 9.1 d) after the experiments; this range is quite close to 28 d, midpoint of the incubation period. Eggs of each species were randomly separated into three groups as the design of the lizard experiment. After measuring the lengths and widths, the eggs were half buried in the wet culture soil in the control treatment. In the FW and SW treatment, eggs were half immersed directly. Each egg was weighed before the treatment and daily in the three continuous days of treatment. This process took three days. In total, 32 eggs of *E. longicaudata*, 30 eggs of *D. swinhonis*, and 9 eggs of *H. frenatus* were used in the egg experiment. There were at least ten individuals of each species in each treatment, except *H. frenatus* (only three) because they were difficult to collect.

We calculated the water loss and the survival rate of the individuals and eggs to determine the effect of treatment. We defined the day that the experiment started as the initial day and the last treatment day as the third day. After the experiments, we calculated the rate of total water loss (RTWL), $(D_0 - D_3 - D_w) / D_0$, where D_0 is the weight of the initial day, D_3 is the weight of the third day during the experiment, and the D_w is the wastes including faeces and shedding skin, which was not used in the egg experiment.

Statistical analysis

We performed four Bayesian models to separately fit the RTWLs/survival rates of the lizards and the RTWLs/hatching rates of the eggs. The RTWLs were fitted by general linear models, and the survival/hatching rates were fitted by logistic regressions. For fitting the RTWLs/survival rates of lizard, we considered the following three factors/covariates: 1) a merging factor which merged species, sex and salinity treatments, 2) scaled mass indexes (SMIs) and 3) the interaction between the two main factors/covariates above. The SMI term was an indicator of body condition accounting for the SVL and weight of lizards and calculated according to Peig & Green [66]. Notably, we excluded the data of the SW group, and all lizards died before day three when fitting the RTWLs, as most of the lizards did not survive before the end of the SW treatment. For fitting the RTWLs and hatching rates of eggs, we considered the following three factors/covariates: 1) a merging factor which merged egg species and salinity treatments, 2) the weights of eggs and 3) the interaction between the two main factors/covariates above. We calculated the Bayes factor (BF) between the models mentioned above and their reduced models, where the weights of the eggs/SMI of the lizards and/or interaction terms were eliminated. Both the interaction between SMI and the sex-stage-treatment and the effect of SMI were highly unsupported in the RTWL and survival rate of the lizards (RTWL:BF < 0.01 in the interaction, BF = 0.02 in SMI; survival rate: BF < 0.01 in the interaction, BF = 0.25 in SMI); similarly, both the interaction between weight and sex-stage-treatment and the effect of weight were highly unsupported in the RTWL and hatching rate of the eggs (RTWL:BF < 0.01 in

the interaction, BF = 0.17 in weight; hatching rate: BF = 0.68 in the interaction, BF = 0.52 in weight); thus, we eliminated these factors in the following results. To achieve multiple comparisons among sex-stages/treatments/species, we performed equivalence testing to compare the survival/hatching rates and RTWLs among sex-stages/treatments/species. We defined the region of practical equivalence (ROPE) as ± 0.2 Cohan's d for comparing RTWLs and as $\pm \log_e(1.2)$ odds ratio for comparing hatching/survival rates.

All Bayesian models were performed via the R package 'brms' [67] under the R framework (version 3.3.1). For each model, ten parallel Markov chain Monte Carlo (MCMC) chains were performed, and 1,000 MCMC samples were collected after 5,000 burn-in iterations per chain. All prior samples were sufficiently weak to cover the potential range of our interesting parameters, and the convergences of the MCMC samples were confirmed visually.

Declarations

Ethics approval and consent to participate

All of the collection, husbandry and treatment procedures followed the Wildlife Conservation Act of Taiwan; the animal use protocols were approved by Institutional Animal Care and Use Committee of the National Museum of Natural Science (license No. 1061701832). All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Authors' contributions

WSH and MHH conceived the idea and designed the experiments. MHH and JYH conducted all experiments. The manuscript was largely written by MHH, JWL, and CPL and then revised by JWL and WSH. CPL analysed the data. All authors read and approved the final manuscript.

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Figures

Figure 1

Current distribution of six lizard species in Taiwan and the historical land connected with the Asian continent. The green patterns represent the distribution of *P. elegans* (A), *E. longicaudata* (B), *D. swinhonis* (C), and three other species (D, E, F). Map G shows the historical land connection between Taiwan and the Asian continent, with labels for 'China' and 'Taiwan'.

(C), *H. frenatus* (D), *A. sagrei* (E) and *E. multifasciata* (F), Taiwan and the adjacent islands with the coastline (G). The long dashed line represents the coastline during the Last Glacial Maximum 26.5 – 18 ka, and the short dashed line represents the coastline before 1.55 Ma.

Figure 3

Rates of total water loss (RTWLs) in six lizard species in Taiwan. RTWLs of *P. elegans* (A), *E. longicaudata* (B), *D. swinhonis* (C), *H. frenatus* (D), *A. sagrei* (E), and *E. multifasciata* (F). Vertical bars present the 95% credible intervals on RTWLs. Alphabet letters above bars presents the results of post hoc comparisons; two groups sharing same letter(s) indicates no significant difference.

Figure 5

Survival rates in three sequent days in six lizard species in Taiwan. Survival rates of *P. elegans* (A), *E. longicaudata* (B), *D. swinhonis* (C), *H. frenatus* (D), *A. sagrei* (E) and *E. multifasciata* (F). Vertical bars

present the 95% credible intervals on the survival rate. Letters above the bars present the results of the post hoc comparisons; two groups sharing the same letter(s) indicates no significant difference.

Figure 7

Rates of water loss (RTWLs, A-C) and hatching rates (D-F) of eggs in three lizard species in Taiwan. RTWLs of eggs of *E. longicaudata* (A), *D. swinhonis* (B), and *H. frenatus* (C). The hatching rates of eggs of *E. longicaudata* (A), *D. swinhonis* (B), and *H. frenatus* (C) in three days. Vertical bars present the 95% credible intervals of RTWLs. Letters above the bars present the results of the post hoc comparisons; two groups sharing the same letter(s) indicates no significant difference. Post hoc comparisons of RTWLs and hatching rates were analysed separately.

Figure 9

Dispersal routes of lizard four species in Taiwan. The orange arrow represents the routes of *Plestiodon* [57]. The brown represents *E. longicaudata* [58]. The blue green represents *Diploderma* [62]. The purple dotted arrow represents the possible route of *E. multifasciata* if it could drift to Green Island and Orchid Island. The dotted line represents the coastline during the Last Glacial Maximum. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.