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First record of carcass use as a refuge in lizards: the phyllodactylid gecko *Homonota darwinii* (Boulenger, 1885) in Argentina

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Darwin's Marked Gecko, *Homonota darwinii darwinii* (Boulenger, 1885), occurs mainly in Argentine Patagonia (Morando et al., 2014) and is the gecko with the southernmost distribution in the world. This small lizard (snout–vent length, SVL = 55 mm) is oviparous and its diet is composed predominantly of arthropods, such as insects and spiders (Kun et al., 2010). It exhibits nocturnal-twilight activity (Ibargüengoytía and Casalins, 2007) and generally uses rocks as daytime refuges (Ibargüengoytía et al., 2007). The selection of these shelters is related principally to thermoregulation (Ibargüengoytía et al., 2007; Aguilar and Cruz, 2010). Thus, the quality of a shelter can guarantee a good level of metabolic maintenance during resting periods and improve performance during nocturnal activity (Aguilar and Cruz, 2010).

Although shelter selection can play an important role in the survival of *H. d. darwinii*, few studies detail its behavioural specifics and microhabitat use (e.g., Aguilar and Cruz, 2010). This lack of information is an important knowledge gap, since understanding the level of plasticity regarding microhabitat use may have implications for the conservation and management of these geckos (e.g., Terán-Juárez et al., 2020; Jins et al., 2022). Among the currently known rest refuges for *H. d. darwinii* are rock fragments, fissures (Aguilar and Cruz, 2010; Breitman et al., 2014), and anthropogenic material (Breitman et al., 2014), such as plastic fishing boxes accumulated on beaches (Hilton Entringer Jr, pers. obs.). However, such elements represent only some of the potential refuges available in Patagonia.

In order to increase the knowledge of the natural history of *H. d. darwinii*, the main goal of this study was to validate the use of carcasses (dead penguins) as refuges by this lizard in two island environments, where the availability of this potential refuge is significantly high compared to other areas. In addition, we aimed to describe and quantify the characteristics of selected carcasses to evaluate their importance as refuges according to their suitability for the species. As *H. d. darwinii* shows a nocturnal-twilight pattern of activity and tends to select refuges according to its size (Ibargüengoytía et al., 2007), we hypothesise that carcasses can be used as daytime resting refuges when they represent the most suitable environmental elements available.

Materials and Methods

Study site. We collected the data on Tova (area 560 ha; 45.0986°S, 65.9981°W) and Tovita (122 ha; 45.1070°S, 65.9621°W), both part of the Tova-Tovita Island Complex, located in the Interjurisdictional Coastal Marine Park *Patagonia Austral* (PIMCPA) and part of the *Patagonia Azul* Biosphere Reserve, Chubut Province, Argentina (APN, 2018). PIMCPA has an area of 104,812 ha and is located in the Patagonian Steppe ecoregion, a vegetation type found on volcanic soil with rocky and sandy components (APN, 2018; Fig. 1A). Several seabird species inhabit the area, including the Magellanic Penguin, *Spheniscus magellanicus*, the most abundant seabird in the region (Yorio et al., 1999) and one reason for an increased general availability of carcasses at this location. The climate of the region is

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considered semi-arid (Beck et al., 2018) with an average annual temperature of 13 °C (APN, 2018). The average accumulated annual precipitation is 227.8 mm. While precipitation can reach maximum daily values of 58.6 mm; precipitation volume is generally less than 5 mm (APN, 2018).

Sampling. In order to test if *H. d. darwinii* used penguin carcasses as refuges, we covered 7 km of linear transects by foot on 18 March 2022. We inspected all carcasses (n = 27) near the transects to confirm the presence of the lizard. Carcasses were classified into three stages: 1- only bones or very fragmented; 2- up to half of the body intact; and 3- more than half of the body intact. When *H. d. darwinii* individuals were detected, the environment was characterised qualitatively by accounting for (1) substrate composition, sandy and/or rocky with or without surface elements (e.g., rock fragments, carcasses); and (2) vegetation parameters, such as the height of bushes and if their relative spacing left a considerable amount of substrate exposed.

We also estimated the availability of possible adequate shelters considering the presence of rocks and penguin

Figure 1. (A) Representation of the sampled environments, with a *Spheniscus magellanicus* carcass located in the centre of the photograph. Size comparison between a carcass occupied by *Homonota darwinii darwinii* (B) and a rocky refuge of the largest area class (C). Photographs by Hilton Entringer Jr.

carcasses (Fig. 1B, C). This was done only on Tova due to the inaccessibility of Tovita. To obtain this estimate, we defined a radius of 20 m from an occupied carcass to create an area of 1257 m². We considered this area adequate based on the reported maximum home range of the similar-sized *H. uruguayensis* (Vaz-Ferreira and Sierra de Soriano, 1961), whose home range has been estimated at 197.9 ± 283.7 m² (Vieira et al., 2020) and *H. borellii* (Peracca, 1897), whose home range was determined to be only 0.50–5.0 m² (Gómez and Acosta, 1998; Godoy and Pincheira-Danoso, 2009). As the actual home range of *H. d. darwinii* remains unknown, our sampling area extrapolated the maximum home ranges of similar-sized geckos to ensure that its possible home range was fully sampled.

Within the defined area, 30 quadrants of area 1 m² were photographed, avoiding vegetated areas and areas that presented only sand without potential shelters. Rock fragments and carcasses present in the quadrants were categorised in ImageJ software (Schneider et al., 2012) according to their respective areas in cm² based on the photographs, disregarding rocks measuring ≤ 10 cm². We classified shelters into six classes according to their size and grouped them into three categories according to their suitability: shelters of 10–25 cm² were considered inadequate since the minimum size to protect the vital parts of a small gecko (ca. 5 cm ventral length) would be approximately 25 cm². Size categories of 25–50 cm² and 50–100 cm² were considered poorly adequate, since the area capable of housing adult specimens in a rectilinear position (ca. 10 cm of total length), regardless of orientation, would be about 100 cm². Finally, areas of 100–150 cm², 150–200 cm², and 200–250 cm² were classified as adequate since these present an area that provides complete protection and also allows for some movement under the shelter. It is noteworthy that the size of potential shelters was used as an indication of suitability for influencing factors related to thermoregulation and also the level of protection against predation (e.g., Vasconcelos et al., 2012). The difference in shelter availability by area category was tested using a Chi-square test (equal expected proportions), considering the availability of each class of area separately. For this test, Yates' continuity correction was applied when the degree of freedom was equal to 1 because this correction generates more adequate values in this context (Zar, 2010). This test was also performed for the inadequate/poorly adequate classes (considering the availability of the three smallest classes together) in comparison to the adequate classes (considering the availability of the three classes with the largest area together). These tests were performed with R environment, v3.5.3. (R CoreTeam, 2021).

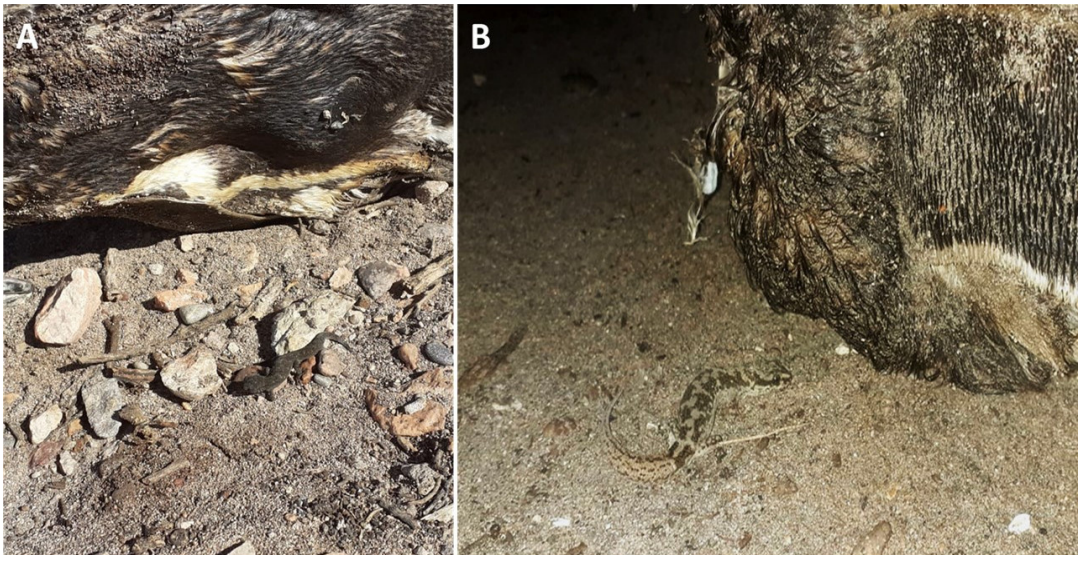

Figure 2. Use of a *Spheniscus magellanicus* carcass as a refuge by *Homonota darwinii darwinii* on Tovita (A) and Tova islands (B), Argentina, highlighting the proximity of the lizard with the most humid region resulting from the contact of the carcass with the soil and the moisture retained in the penguin's feathers. Photographs by Hilton Entringer Jr.

Results

Among the 27 carcasses inspected (only bones or very fragmented = 8; up to half of the body intact = 10; more than half of the body intact = 9), two (7.4%) were used as a refuge by adults of *H. d. darwinii.* These carcasses were in the initial stages of decomposition, with intact skin and plumage. The temperature in the island complex varied between 6–36 ºC during the sampled period. The first carcass use was recorded on Tovita Island at 12:24 h (temperature = 35 °C), and the lizard was located in the most humid region between carcass and soil, an area of about 243 cm² (Fig. 2A). The site had no rocky outcrops, and the substrate was predominantly sandy and exposed (Fig. 1A). Around the carcass, rock fragments, which were predominantly elliptical and flattened, were scattered on the sand (Fig. 1C). The surrounding vegetation consisted of scattered shrubs (height ca. 50 cm; Fig. 1A). At this location, penguin carcasses were the largest shelters available. The second carcass was found on Tova Island at 14:12 h (temperature = 21 °C), in a similar environment as the first record. In this location, the lizard was found under a carcass 235 cm² area (Fig. 2B). It is noteworthy that when the carcass was lifted for inspection, the exposed lizard tried to take refuge in areas of the carcass that remained in contact with the ground. This carcass was inspected again on the same day at 19:56 h (temperature = 15 °C) and on the following morning at 08:25 h (temperature = 12 °C), with the individual present in the evening and absent in the morning.

Of the 234 evaluated shelters around the carcasses, 66.7% (n = 156) were considered inadequate (< 25 cm²) and 27.8% (n = 65) were considered poorly adequate (25–50 cm², 22.6%, n = 53; 50–100 cm², 5.1%, n = 12). Adequate shelters represented 5.6% (n = 13) of the total evaluated (100–150 cm², 3.0%, n = 7; 150–200 cm², 0.4%, n = 1; 200–250 cm², 2.1%, n = 5). Among the largest shelters, 60.0% (n = 3) were penguin carcasses with an area of 203–235 cm², while the two largest rocks represented 40.0% of potential shelters with areas of 216 cm² and 244 cm². The availability of shelters differed between the area categories, both when compared individually by size ($\chi^2 = 467.641$; $df = 5$; $p \leq 0.001$) and when compared according to their suitability between inadequate/poorly adequate (94.4% of shelters, $n = 221$) and adequate categories (5.6%; $n = 13$) ($\chi^2_{Yates} = 183.115$; $df = 1$; $p \leq 0.001$).

Discussion

Our results suggest that shelters selection by *H. d. darwinii* may be related to shelter area since the species utilised more intact penguin carcasses, which were among the largest available shelters even though these generally were less available than rocks. The hypothesis that geckos selected refuges by size is supported by the finding that they did not use smaller shelters in other localities, as observed in continental populations (Aguilar and Cruz, 2010). In addition, adults of other saxicolous geckos from arid and insular environments, such as the São Vicente Wall Gecko, *Tarentola substituta* Joger, 1984, also select larger rocks as refuges even when these may be less available (Vasconcelos et al., 2012; Pereira et al., 2019). Thus, the presence of shelters of adequate size may contribute to the maintenance of lizard species in arid island environments.

In lizards, thermal properties are among the factors that induce the selection of specific habitats (Ibargüengoytía et al., 2007; Aguilar and Cruz, 2010; Vasconcelos et al., 2012). For *H. d. darwinii* specifically, occupying thermally adequate rest refuges allows the species to fulfil its physiological and metabolic needs when inactive during the day, which can result in better performance during nocturnal activity (Aguilar and Cruz, 2010). Thus, the selection of good shelters can be especially important in the study region since Patagonian Steppe areas can present extreme thermal variations (e.g., Aguilar and Cruz, 2010).

Protection against predation should also be considered, which is an important function of shelters and can define their selection by geckos (Schlesinger and Shine, 1994; Shah et al., 2004). Thus, lizards are expected to select refuges in order to balance thermoregulation with predation risk (Vasconcelos et al., 2012). In the case of places without terrestrial predators that can easily overturn small shelters, smaller refuges may be used (Vasconcelos et al., 2012). Although there are no native terrestrial predators in the study area, there is an established house cat population (Udrizar Sauthier et al., 2017) that was introduced at least 25 years ago (Schiavini et al., 2005), that predates upon lizards in the Tova-Tovita complex (Hilton Entringer Jr and Georgina Squartini, pers. obs.). Hence the selection of larger shelters may represent an anti-predation strategy, both for protection and for escape. However, it is also important to consider that the use of carcasses as a refuge could furthermore facilitate lizard's predation by aerial scavengers in the islands, since penguin carcasses can attract birds such as the *Caracara plancus* Miller,

1777 (Hilton Entringer Jr and Gabriela S. Blanco, pers. obs. in continental areas of the PIMCPA), which can also consume lizards (Vargas et al., 2007).

Moisture is another factor influencing roost selection by lizards, as observed for the geckos *Eublepharis macularius* (Blyth, 1854) and *Sphaerodactylus notatus* (Baird, 1859) (Allen et al., 2015; Craioveanu et al., 2017). This characteristic of shelters may be important for geckos because water restriction negatively affects their physiology, including their thermoregulation (Chukwuka et al., 2020). Therefore, moisture provided by the carcasses in the midst of a predominantly arid matrix, as observed in the present study, may also influence the selection of this type of refuge by *H. d. darwinii*.

Until now, reports of carcass use by lizards were based on trophic interactions, the most frequent being related to direct consumption of carcasses, as observed for *Salvator merianae* (Duméril & Bibron, 1839), *Podarcis lilfordi* (Günther, 1874), and *Varanus komodoensis* Ouwens, 1912 (Bull et al., 2010; Sazima and D'Angelo, 2013; Pérez-Cembranos et al., 2016). Furthermore, carcasses in general, including those of penguins, have great potential to attract insects that can be used by lizards as food (Tréhen et al., 1985; Chown and Language, 1994; Abernethy et al., 2017; Mashaly et al., 2019; Andrade-Herrera et al., 2020). In general, species tend to occupy the most favourable environments for food acquisition to optimise energy gain (Stephens et al., 2007). Thus, lizards can also be attracted to carcasses because of prey capture potential, as observed for *P. lilfordi* (Pérez-Cembranos et al., 2016). Since insects are predominant in the diet of *H. d. darwinii* (Kun et al., 2010), the greater availability and/or ease of capturing prey may also contribute to the selection of carcasses by this lizard. With this, we also suggest that individuals can be attracted to the carcass during foraging activity. If they represent good refuges, especially in places where adequate shelter is scarce, these geckos may use its food source as shelter, optimising energy by avoiding movement between resting and feeding areas.

These first records of the use of *S. magellanicus* carcasses as refuge by *H. d. darwinii* contribute to the natural history knowledge of the species, particularly their habitat use. Overall, we suggest that the use of carcasses as a refuge may be mainly related to thermoregulation, but in balance with defence against predation, desiccation, and food acquisition, although these associated factors should be further investigated. In addition, the records presented here also represent the first records relating the use of carcasses as a refuge for lizards in general, which suggests that other species of this group may also have the potential to exhibit this behaviour. The present study further emphasises the importance of migratory seabirds as penguins in the Patagonian ecosystem, as their carcasses not only offer extra food for scavengers, but also act as shelter for smaller species. And thereby highlights the importance for conservation action of such charismatic species, especially those considered as "umbrella species" that can favour the protection of other species of a given location, region, or ecosystem. Finally, our contribution demonstrates that more attention should be given to alternative shelters in field studies, since species considered saxicolous, such as *H. d. darwinii*, can also take refuge in other environmental elements.

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