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Cover: Adult male of *Pristimantis relictus* from northeastern Brazil.
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Diet composition of *Caretta caretta* (Testudines: Cheloniidae) in the Gulf of Gabès, southern Tunisia

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Abstract

Diet composition of *Caretta caretta* (Testudines: Cheloniidae) in the Gulf of Gabès, southern Tunisia. Investigating fundamental biological aspects, such as the feeding habits of long-distance migratory marine animals like sea turtles, presents significant challenges. These studies are crucial for identifying the feeding grounds and preferred prey of these turtles, thus providing valuable insights to inform habitat protection and management decisions. We analyzed the digestive tract contents of 132 *Caretta caretta* stranded and/or accidentally captured along the coast of the Gulf of Gabès from 2004 to 2010. Food items were analysed using the Relative Importance Index (IRI), the Food Index of Geistdoerfer (Q), and the Main Food Index (MFI) of Zander. We also categorized the litter and calculated its frequency of occurrence. The loggerhead diet consisted of 46 species from various zoological groups, reflecting their flexible distribution across both pelagic and benthic neritic zones. We noted a common feeding pattern for the species, with bottom-dwelling prey common in shallow environments. Molluscs, arthropods, and poriferans constituted the main prey, while tunicates and fishes were frequent secondary prey. Echinoderms and algae are accessory secondary prey and second-order additional prey, respectively. Molluscs and arthropods were ingested mainly during spring and summer, while tunicates and poriferans were ingested during autumn and winter months. Turtle size had no effect on dietary diversity or biomass percentage. This study emphasizes the value of dietary analysis in uncovering the feeding ecology and habitat use of loggerhead turtles in the Gulf of Gabès. Protecting and managing this region, and ensuring the presence of trophic resources, could be crucial for maintaining loggerhead turtle populations and enhancing their survival in Tunisian waters.

Keywords: Digestive tracts, Feeding habits, Loggerhead turtles, Mediterranean Sea.

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Resumo

Composição da dieta de *Caretta caretta* (Testudines: Cheloniidae) no Golfo de Gabès, sul da Tunísia. A investigação de aspectos biológicos fundamentais, como os hábitos alimentares de animais marinhos migratórios de longa distância, como as tartarugas marinhas, apresenta desafios significativos. Esses estudos são essenciais para identificar os locais de alimentação e as presas preferidas desses quelônios, fornecendo assim informações valiosas para embasar decisões de proteção do habitat. Analisamos o conteúdo do tubo digestivo de 132 espécimes de *Caretta caretta* encalhadas e/ou capturadas acidentalmente ao longo da costa do Golfo de Gabès, de 2004 a 2010. Os itens alimentares foram analisados usando o Índice de Importância Relativa (IRI), o Índice Alimentar de Geistdoerfer (Q) e o Índice Alimentar Principal (MFI) de Zander. Também categorizamos o lixo e calculamos sua frequência de ocorrência. A dieta da tartaruga-cabeçuda consistiu de 46 espécies de vários grupos zoológicos, refletindo sua distribuição flexível em zonas neríticas pelágicas e bentônicas. Observamos um padrão de alimentação comum para as espécies, com presas que habitam o fundo do mar, comuns em ambientes rasos. Moluscos, artrópodes e poríferos constituíram as principais presas, enquanto tunicados e peixes foram presas secundárias frequentes. Os equinodermos e as algas são presas secundárias acessórias e itens adicionais de segunda ordem, respectivamente. Moluscos e artrópodes foram ingeridos principalmente durante a primavera e o verão, enquanto tunicados e poríferos foram ingeridos durante os meses de outono e inverno. O tamanho da tartaruga não teve efeito sobre a diversidade da dieta ou a porcentagem de biomassa. Este estudo enfatiza o valor da análise da dieta para descobrir a ecologia alimentar e o uso do habitat das tartarugas-cabeçudas no Golfo de Gabès. Proteger e gerenciar essa região e garantir a presença de recursos tróficos pode ser crucial para manter as populações de tartarugas-cabeçudas e aumentar sua sobrevivência nas águas da Tunísia.

Palavras-chave: Hábitos alimentares, Mar Mediterrâneo, Tartarugas-cabeçudas, Tubos digestivos.

Introduction

Currently, all sea turtle species except the Australian Flatback, *Natator depressus* (Garman, 1880), are included in the IUCN Red List as Endangered or Vulnerable (Godfrey and Godley 2008, Wallace *et al.* 2011, IUCN 2024). The conservation of these chelonians presents a multifaceted challenge due to various factors, including their biology, life cycle, and their primarily marine habitat (Lutcavage *et al.* 1997, Rees *et al.* 2013), which limits direct observations and research opportunities, especially regarding their foraging habits and diet (Tomás *et al.* 2001). Because of this, strandings and bycatch provide scientists with a wealth of ecological and biological data on sea turtles, such as location and quality of their feeding grounds, use of trophic resources, and how human activity affects their habitats (Ullmann and Stachowitsch 2015, UNEP/MAP - SPA/RAC 2019). Comprehensive knowledge of each of these elements is crucial for effective population management (Bjorndal 1997).

Loggerhead sea turtles, *Caretta caretta* (Linnaeus, 1758), inhabit temperate and tropical regions of the Atlantic, Pacific, and Indian Oceans, and the Mediterranean Sea. As adults, this species migrates long distances between foraging regions and nesting beaches (Dodd 1988, Plotkin and Spotila 2002) with the majority of nesting occurring in the western rims of the Atlantic and Indian Oceans. In the Mediterranean, most nesting occurs in Greece, Turkey, Cyprus, and Libya (Margaritoulis *et al.* 2023). Adults and juveniles are present in the entire basin, concentrated in foraging areas along the North Adriatic and Central Mediterranean continental shelfs, with lower abundance in the southern Ionian and in the area between Sicily and Tunisia (Mancino *et al.* 2022).

The life cycle of *C. caretta* involves distinct ontogenetic habitat shifts that coincide with their growth stages (Casale *et al.* 2008). These shifts in habitat preference are related to changes in feeding strategies and diving capabilities as the turtles mature (Fouda 2021, Mariani *et al.* 2023). When hatchlings reach the sea, the oceanic phase begins, and they migrate to the open sea where they spend most of their juvenile stage. During this phase, *C. caretta* typically has a curved carapace length (CCL, notch to tip; Bolten 1999) of up to approximately 59.9 cm (Bjorndal *et al.* 2000, Tomás *et al.* 2001). Due to their limited diving capability, juvenile turtles primarily feed on pelagic prey found in the open ocean (Bolten 2003). As the turtles grow, they enter a transitional phase leading to the subadult stage (CCL range of 60–69.9 cm). During this phase, *C. caretta* utilizes both oceanic and neritic habitats (Palmer *et al.* 2021). They start to explore and inhabit more benthic environments, gradually drawing closer to neritic areas (Casale *et al.* 2008). Once they reach the adult stage and become sexually mature (CCL > 70 cm), they transit to neritic areas (Tomás *et al.* 2001, Casale and Margaritoulis 2010). In these shallower coastal areas, they predominantly feed on benthic organisms (Casale *et al.* 2011, Lazar *et al.* 2011). These habitat shifts observed throughout the life cycle reflect their changing feeding habits and adaptation to different environments as they grow and develop (Casale *et al.* 2008). Understanding these ontogenetic habitat shifts and corresponding changes in feeding preferences is crucial for conservation efforts and the management of critical habitats that are essential for the various life stages of loggerhead sea turtles.

The diet of *C. caretta* in the Mediterranean regions has been directly studied through analyses of feces and stomach contents (e.g. Benhardouze *et al.* 2021, Baldi *et al.* 2023, Mariani *et al.* 2023), and indirectly through stable isotope analysis (e.g. Blasi *et al.* 2018, Haywood *et al.* 2020, Cardona *et al.* 2024). This species is considered opportunistic carnivorous, and the presence of fast-moving prey, such as fish and cephalopods, has raised the question of the potential role of fishing discards in the turtle diet (Tomás *et al.* 2001, Casale *et al.* 2008, Baldi *et al.* 2023, Cardona *et al.* 2024).

The African continental shelf off Tunisia, characterized by favorable geomorphological, climatic, and oceanographic conditions, sustains one of the most productive ecosystems in the western Mediterranean Sea (Ben Salem *et al.* 2002, Hattab *et al.* 2013). This is the second widest continental shelf area in the region, and it is one of the most important neritic feeding grounds for juveniles and adults of *C. caretta* in the Mediterranean Sea (Casale and Margaritoulis 2010, Bradaï *et al.* 2020). Prior research conducted on *C. caretta* in the area had constraints such as a limited number of samples or a concentration on specific turtle size classes, which restricted a comprehensive understanding of feeding habits of these populations (Laurent and Lescure 1994, Bradaï 2000). The current study offers more recent information on the feeding habits of loggerhead sea turtles in the Gulf of Gabès using a more robust sample.

Materials and Methods

Study Area

This study was carried out in the Gulf of Gabès, located in the central Mediterranean Sea (33°–35° N and 10°–12.5° E) extending from Ras Kaboudia (Chebba) to the Tunisian–Libyan frontier (Figure 1). The region hosts one of the largest meadows of *Posidonia oceanica* (L.) Delile in the area (El Zrelli *et al.* 2020), which serves as a nursery, as well as feeding and breeding habitat for many marine species (Bradaï *et al.* 2004, Enajjar *et al.* 2015). It is considered one of the Mediterranean’s most productive ecosystems (Papaconstantinou and Farrugio 2000). The seafloor is predominantly soft, resulting in the prevalence of demersal trawling (Abdou *et al.* 2018). Catches in the Gulf are dominated by cephalopods, shrimps, and demersal finfish (Sparidae and mullets) (Hattab

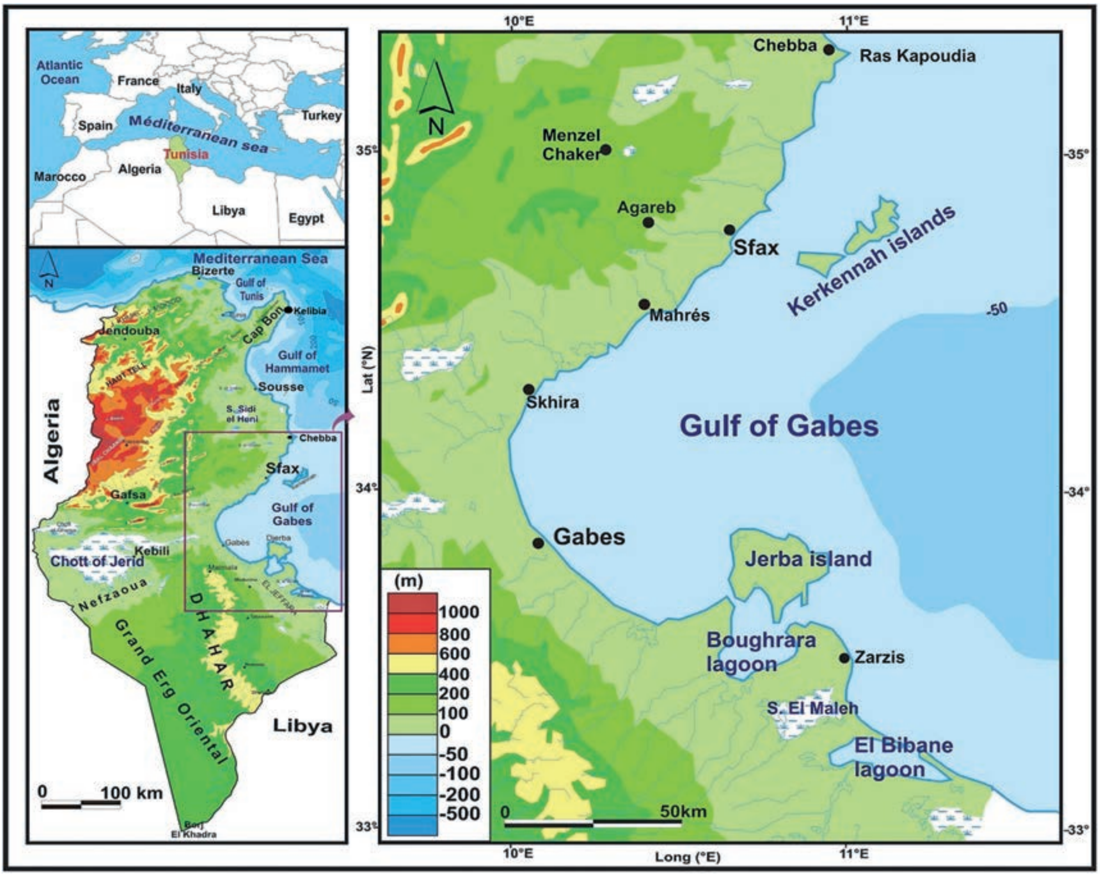

Figure 1. Localization of the Gulf of Gabès (map adapted from Béjaoui *et al.* 2019).

et al. 2013). Due to global change, non-native species are invading the Gulf of Gabès (Bradaï *et al.* 2004). Several migrants from the Red Sea and the Atlantic Ocean are either permanent residents or sporadically observed (Ben Amor *et al.* 2016, Béjaoui *et al.* 2019).

Sample Collection

In Tunisia, the National Institute of Marine Sciences and Technologies (INSTM) has monitored sea turtle and cetacean strandings since 2004 through the National Stranding Network (RNE) (Karaa *et al.* 2012). A national coordinator oversees the network, which consists of three regional monitoring groups: one covering the northern coast (from the Algerian border to Kelibia), another in the central region (from Kelibia to Chebba), and a third in the south (in the Gulf of Gabès) (Figure 1).

RNE members survey beaches and respond to information from NGOs, fisheries authorities, and coast guards in order to investigate stranded sea turtles. The network also records cases of sea turtle bycatch (UNEP/MAP - SPA/RAC 2020). All collected data, including stranding/capture date, location, and sex, are stored in a dedicated database. Each year, INSTM compiles these records into a report that is shared with RNE partner administrations and made available to the public upon request.

We analysed the diet of *C. caretta* in the Gulf of Gabès between October 2004 and June 2010. During this period, we examined the digestive tracts of 132 turtles. Seasonal sampling included 24 turtles in winter and fall ($N = 10$ and 14, respectively) and 108 turtles in spring and summer ($N = 72$ and 36, respectively). The highest number of turtles sampled was in May (56 turtles).

The sampled turtles included 111 dead stranded turtles from different locations (57 in Gabès, 22 in Jerba Island, 20 in Zarzis, five in Kerkennah Island, six in Sfax, and one in Chebba; Figure 1) as well as 21 individuals accidentally caught in benthic trawls off Sfax and discovered dead upon arrival at INSTM. We performed necropsies at the INSTM Center in Sfax following the protocol described by Work (2000). During necropsies, we isolated the contents of the esophagus, stomach, and intestines, rinsed them with fresh water, and sieved them using a 1 mm mesh.

Data Collection

We identified prey items to the lowest possible taxonomic level and estimated their numbers when feasible. After drying samples at approximately 60°C for 24 hours, we measured their dry weight to the nearest tenth of a gram. For consistency, we limited our analysis to macroscopic items (> 1 mm). Prey identification relied on distinguishable remains such as fish bones, cephalopod beaks, prosobranch gastropod opercula, and decapod chelipeds.

To classify the digestive contents, we used a binocular magnifying glass and referred to multiple identification guides (Riedl 1963, Fischer *et al.* 1987). When digestion was too advanced for precise species identification, we assigned prey items to broader taxonomic categories, such as Family, Order, or Class.

We categorized digestive contents of the turtles into three categories based on their origin and state: (1) prey, which includes the remains of organisms that turtles have captured either alive or dead; (2) remains that were likely inadvertently captured by the turtles while they were foraging for food (non-prey remains); and (3) debris.

Data Analysis

The quantitative analysis of prey included estimates of the following parameters usually used in diet studies and involving the following coefficients:

Percentage of prey item in number (%N).—This metric represents the percentage ratio between the number of individuals of a prey item i (Ni) and the total number of prey (Nt): $\%N = 100 \times (Ni / Nt)$.

Percentage of prey item in weight (%W).—Percentage ratio of the weight of individuals_of prey item i (wi) and the total weight of prey (Wt): $\%\mathrm{W} = 100 \times (wi / Wt)$.

Food coefficient or prey (Q) (Geistedoerfer 1975).—$\mathrm{Q} = \%N \times \%\mathrm{W}$ where: $\%N$ and $\%\mathrm{W}$ represent the percentage of prey in number and in weight, respectively.

Frequency of occurrence of prey item (%F).—This measure represents the ratio, expressed in percentage, of the number of observations of a specific prey item i (Ni) and the total number of full digestive tracts analyzed (Nt): $\%F = Ni / Nt$.

Main Food Index (MFI) (Zander 1982).—The MFI for each food item integrates three indices: $\%N$, $\%F$, and $\%\mathrm{W}$ and is calculated using the formula: $\mathrm{MFI} = [\%\mathrm{W} \times (\%N + \%F) / 2]^{1/2}$.

Index of relative importance (IRI).—This index was calculated to indicate the importance of each prey (Pinkas *et al.* 1971): $\mathrm{IRI} = \%\mathrm{Fi}\,(\%\mathrm{Wi} + \%N\mathrm{i})$, where i represents a specific prey item, %*F* is its frequency of occurrence, and %W and %*N* represent its proportion of contents in the digestive tracts by weight and by number, respectively. The IRI values were converted to a percentage to facilitate comparisons between prey items (Cortés 1997).

Percent index of relative importance (%IRI).—This index provides a standardized measure for dietary analysis. It is calculated using the following equation: %IRIi = 100 × (IRIi/IRIt), where %IRIi is the Percent Index of Relative Importance for prey item *i*, and IRIt represents the total of all Indexes of Relative Importance values for prey.

The methods of Geistdoerfer (1975) and Zander (1982) are used to categorize prey. According to Zander (1982), prey are classified into four groups: essential prey (MFI > 75), primary prey (51 ≤ MFI ≤ 75), secondary prey (26 ≤ MFI ≤ 50), and accessory prey (MFI < 26). The dietary coefficient of Geistdoerfer (1975) divides the prey into three categories, each subdivided into two sub-categories using both food coefficient Q and frequency index %*F* as following: (1) Main prey Q > 100, preferential %*F* > 30, occasional %*F* < 30; (2) Secondary prey: 10 < Q < 100, frequent %*F* > 10, accessory %*F* <10; and (3) Additional prey: Q <10, first order %*F* >10, second order *F* % <10.

Diet Shifts

We used a non-parametric MANOVA to analyze feeding variation. We applied a two-factor design to test the null hypothesis that diet did not differ between sexes and predator size (CCL).

We conducted statistical analyses based on the prey categories. To evaluate potential dietary differences with size, we divided the turtles into two size classes according to their life stages (Casale *et al.* 2008): class I: post-pelagic juveniles (PPJ) (CCL > 35 cm) and class II: adults (CCL ≥ 70 cm).

We considered the number of prey in each category as the dependent variables, and defined sex (Female or Male) and size class (I, II) as factors. To test differences in diet, we applied the multivariate *F* value (Wilks' lambda), which compares the error variance/covariance matrix with the effect variance/covariance matrix.

Results

Sample Collection

We examined the diet of loggerhead turtles in the Gulf of Gabès from October 2004 to June 2010. During this period, we analyzed 132 digestive tracts, of which 91 contained feeding remains. Of these turtles, 70 turtles were found stranded, while 21 were accidentally captured dead by bottom trawls. Among the stranded turtles, one showed signs of boat strike, three had hooks in their intestines, and 66 exhibited no apparent cause of mortality.

The turtles studied (*N* = 91; mean = 58.32 cm; SD = 9.14) varied in size, with the smallest with a CCL of 28 cm, while the majority (*N* = 90; 99%), had a CCL greater than 35 cm. Of these, 76 turtles (83.52%) were classified as post-pelagic juveniles (PPJ), and 14 (15.38%) were adults. The largest turtle recorded had a CCL of 77.5 cm. Most of these turtles were sampled in spring and summer and were predominantly found in early decomposition (*N* = 37; 40.65%) or decomposed (*N* = 20; 21.97%); 34 turtles (37.36%) were found freshly dead (Figures 2 and 3).

Dietary Composition

Dietary analyses showed that *C. caretta* feed on a variety of prey (Figure 4). Of analyzed turtles, 66% (*N* = 60) consumed up to 20 different prey items (Figure 5).

Undigested organic matter.—Loggerhead turtles showed a high dietary diversity, with 46 species of various zoological groups (Table 1). The total weight of the 2520 prey items was

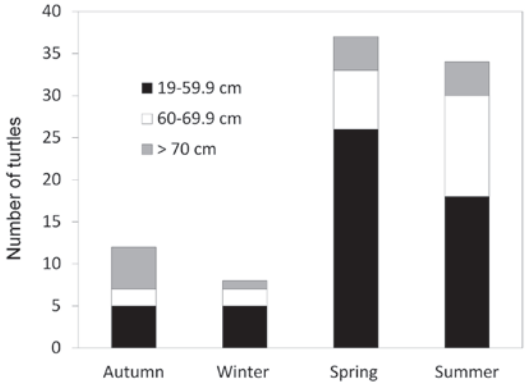

Figure 2. Curved Carapace Length (CCL) frequency histogram of the sampled turtles according to the seasons.

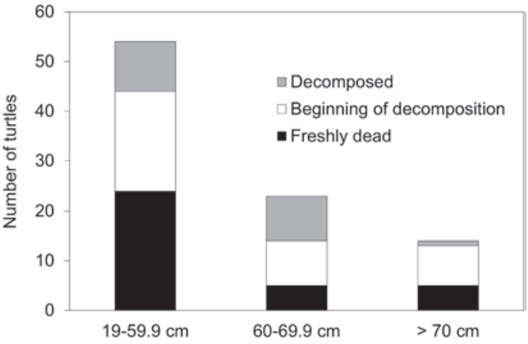

Figure 3. Curved Carapace Length (CCL) frequency histogram of the sampled turtles according to their stages of decomposition.

17.28218 kg, with an average of 27.7 prey items per digestive tract. Notably, two adult turtles had 313 and 278 individuals of the sponge *Chondrosia reniformis* Nardo, 1847, each weighing over 2 kg, in their digestive tracts. These turtles had carapace lengths (CCL) of 77.5 cm and 72 cm and were stranded in May and November 2008, respectively.

Molluscs and arthropods served as preferred primary prey (Q > 100 and *F*% > 30%), while porifera were occasional prey (Q > 100 and *F*% < 30%) (Table 1). As secondary prey (10 < Q < 100), we found tunicates, fish, and echinoderms at the following frequencies: 12.1 (*N* = 11), 18.7 (*N* = 17), and 7.7 (*N* = 7) (Table 1), respectively. Molluscs and arthropods represented the primary food sources, with average masses of 27.7 g and 43.59 g, corresponding frequencies of 60.4% and 49.5%, and %IRI of 39.4% and 30.5%. Those taxa were primary represented by the cuttlefish, *Sepia officinalis* Linnaeus, 1758, (%*F* = 33), the gastropod, *Hexaplex trunculus* (Linnaeus, 1758) (%*F* = 22), the crab, *Eucrate crenata* (de Haan, 1835) (%*F* = 19.8), and the spot-tail mantis shrimp, *Squilla mantis* (Linnaeus, 1758) (%*F*= 15.4).

The most recorded species of molluscs was the cuttlefish, *Sepia officinalis.* This species served as food source for 30 turtles (CCL ranged from 28 cm to 73 cm; mean = 54.94; SD = 10.06). The sample included two mature turtles (CCl = 72 cm and 73 cm), which stranded in Gabès in September 2007 and August 2008, respectively, and two juveniles (CCL = 28 cm and 37 cm). The first juvenile turtle was stranded in Gabès in July 2008 and had a cuttlefish beak weighing 3.15 g in its stomach. The second turtle, which stranded in Chebba in June 2008, was found to have the remains of two cuttlefish totaling 30.5 g in its tract. The second most recorded molluscs, the gastropod, *H. trunculus,* was recorded from 20 turtles across various seasons of the year (4 in autumn, 2 in winter, 8 in spring, and 6 in summer). Concerning the two turtles sampled during winter, one was a subadult (CCL = 58 cm) stranded in January 2005; the other one was an adult female (SCCL = 77 cm) stranded in December 2007. The gastropod, *H. trunculus,* was associated with marine phanerogams, *Posidonia oceanica* or *Cymodocea nodosa* (Ucria) Asch., in both cases (subadult and adult female *C. caretta*).

Several fish species with distinct habitat preferences were identified: *C. caretta* fed on benthic prey [e.g., Signatidae, *Hypocampus hippocampus* (Linnaeus, 1758) and Mullidae, *Mullus* sp.], pelagic prey (e.g., Clupeidae, *Sardinella aurita* Valenciennes, 1847) and prey

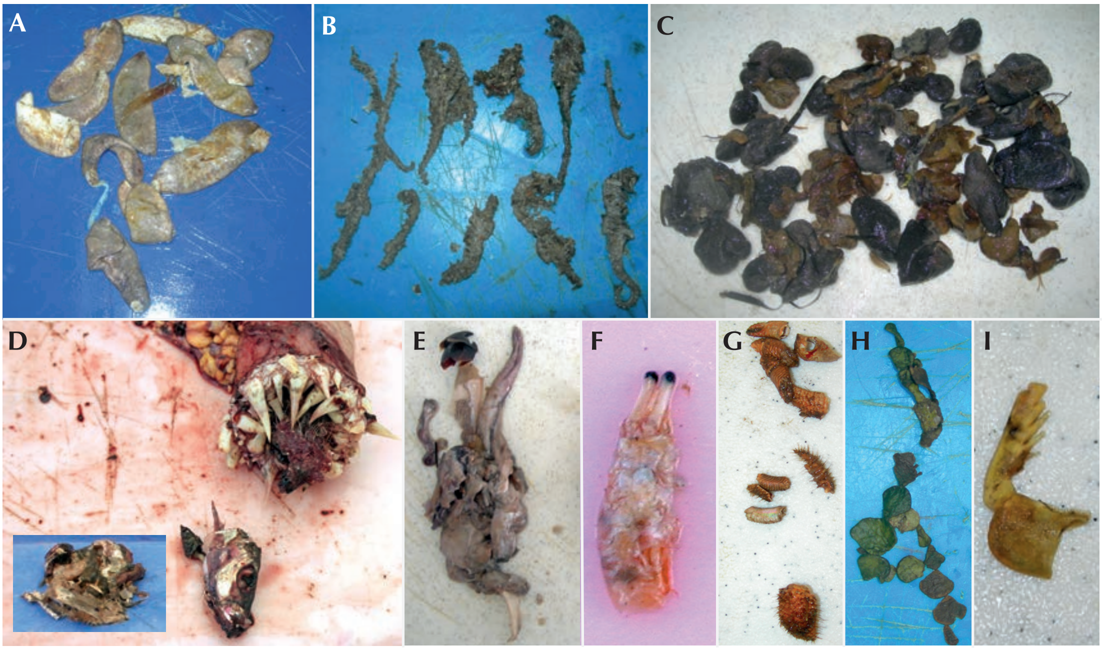

Figure 4. Examples of prey ingested by *Caretta caretta* in the Gulf of Gabès: (**A**) *Holothuria polii* (white spot cucumber); (**B**) *Hypocampus hypocampus* (Short snouted seahorse); (**C**) *Chondrosia reniformis* (Leather-Sponge); (**D**) *Sardinella aurita* (Gilt sardine); (**E**) *Sepia officinalis* (Common cuttlefish); (**F**) *Pagurus* sp. (Hermit crabs); (**G**) *Pilumnus hirtellus* (bristly crab); (**H**) *Halimeda tuna* (sea cactus); (**I**) Chela of *Squilla mantis* (Spot-tail Mantis Shrimp).

with a demersal distribution [e.g., Sparidae, *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817)]. In our samples, syngnathid fish were rare, found in the digestive tract of only two turtles stranded during the spring season—one subadult (CCL = 59 cm) and one adult female (CCL = 70 cm)—both of which had 10 and 12 individuals of *H. hippocampus* in their diet, respectively.

Plant remains mainly consisted of the green algae, *Halimeda tuna* (J. Ellis & Solander) J. V. Lamouroux, *C. nodosa,* and *P. oceanica.* These algae, found in the digestive tract of 25 turtles (%*F* = 27.5; Table 1), were still pigmented and appeared undigested. Similarly, porifera, *C. reniformis* and *Tethya* sp., were found predominantly intact within the gastrointestinal

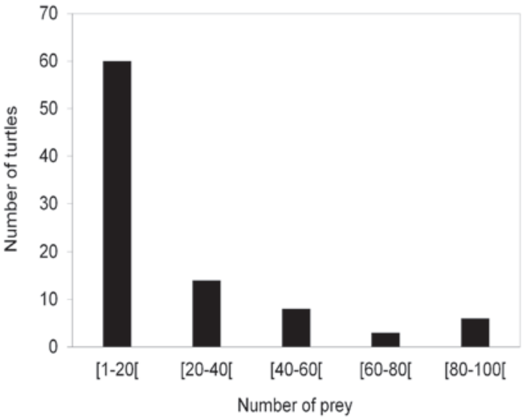

Figure 5. Frequency distribution of number of prey items versus number of turtles presenting food in their digestive tract.

Table 1. Classification of prey of *Caretta caretta* (Loggerhead Sea Turtle) in the Gulf of Gabès in categories according to Zander (1982) and Geistdoerfer (1975); *one ovisac of Rajidae was found in the stomach of a subadult turtle (CCL = 58 cm) in spring 2005 in Sfax region; %*F*: Frequency of occurrence of prey item; %W: Percentage of prey item in weight; %*N*: Percentage of prey item in number; %IRI: Percentage index of relative importance: %Q: food coefficient; MFI: Main Food Index.

Taxon/Prey types and their frequencies of occurrence	**%F**	**%W**	**%N**	**%IRI**	**%Q**	**MFI**	**Prey categories (Zander 1982)**	**Prey categories (Geistdoerfer 1975)**
Molluscs	60.4	14.6	25.6	39.4	372.76	67.63	Main prey	Preferred main prey
Gastropoda: *Hexaplex trunculus* (22), *Turritella* sp. (1.1), *Turritella communis* (1.1), *Cerithium scabridum* (3.3), *Cerithium vulgatum, Bittium reticulatum* (1.1), Unidentified gastropods (8.79); Bivalvia: *Pinctada radiata* (5.49), *Mactra corallina* (1.1), *Cerastoderma glaucum* (1.1), *Cardium* sp. (1.1), *Ostreola stentina* (1.1), *Ostreola* sp. (1.1), *Cerithium vulgatum* (1.1), unidentified Bivalvia (3.3); Cephalopoda: *Octopus vulgaris* (1.1), *Sepia officinalis* (33), unidentified Cephalopoda (6.58)								
Arthropoda	49.5	23	15.1	30.5	347.3	92.20	Essential prey	Preferred main prey
Crabs: *Eucrates crenata* (19.8), *Maja squinado* (5.49), *Libinia dubia* (1.1), *Maja* sp. (8.79), *Parthenope anguilifrons* (3.3), *Pilumnus hirtellus* (1.1), unidentified crab (7.69); Shrimps: *Metapenaeus monoceros* (1.1); *Melicertus kerathurus* (3.3), *Squilla mantis* (15.4); Hermit crab: *Pagurus* sp. (2.2); Balanomorph cirriped: *Balanus perforatus* (2.2)								
Porifera	16.5	43.2	38	21.7	1641.6	159.4	Essential prey	Occasional main prey
Chondrosia reniformis (12.1), *Tetya* sp. (7.69)								
Bony fishes	18.7	3.5	3.2	2	11.2	8.27	Accessory prey	Frequent secondary prey
Diplodus vulgaris (1.1), *Sardinella aurita* (3.3), *Mullus* sp. (1.1), *Hypocampus hippocampus* (2.2), unidentified fish (13.2)								
Tunicates	12.1	7.5	7.3	2.9	54.75	16.48	Accessory prey	Frequent secondary prey
Pyura dura (6.59), *Microcosmus vulgaris* (8.79)								
Echinodermata	7.7	5.4	4.6	1.3	24.84	9.51	Accessory prey	Accessory secondary prey
Paracentrotus lividus (2.2), *Holothuria polii* (3.3), unidentified echinoderms (2.2)								
Nemathelminthes	2.2	0.1	2	0.1	0.2	0.07	Accessory prey	Second-order additional prey
Tonaudia tonaudia (1.1), *Kathlania leptura* (1.1)								
Platyhelminthes	2.2	0.01	1.79	0.1	0.018	0.007	Accessory prey	Second-order additional prey
Diaschistorchis pandus (2.2)								
Algae	27.5	2.14	1.79	1.7	3.83	5.77	Accessory prey	Second-order additional prey
Caulerpa prolifera (1.1), *Coduim bursa* (1.1), *Lithophyllum racemus* (2.2), *Phymatolithon calcareum* (1.1), *Halimeda tuna* (7.69), *unidentified algues* (2.2), *Cymodocea nodosa* (13.2), *Posidonia oceanica* (13.2)								
Elasmobranchs*	1,1	0.01	0.04	0	0	0.004	Accessory prey	Second-order additional prey
Ovisac of *Raja* sp. (1.1)								

system of the necropsied turtles. Fifteen turtles (%*F* = 16.5; Table 1) had sponges in their diet.

This study detected 3 non-native species in the digestive contents of *C. caretta:* the pearl oyster, *Pinctada radiata* (Leach, 1814); the spider crab, *Libinia dubia* H. Milne-Edwards, 1834; and the blunt-spined euryplacid crab, *Eucrate crenata*. Additionally, two balanomorph barnacles (*Balanus perforates* Bruguière, 1789), one of which was affixed to a pearl oyster (*P. radiata*), were found in the undigested organic remains of two turtles: one subadult (CCL = 66 cm) and one adult (CCL = 72 cm).

Non-organic matter (Debris).—Thirteen of the 132 turtles analyzed (9.85%) had anthropogenic debris, such as soft plastics, ropes, wood, and fish hooks, while three turtles (2.32%) had sandy debris (Tables 2 and 3) in their digestive tracts. With the exception of fish hooks,

Table 2. Type and occurrence of marine debris ingested by *Caretta caretta* (*N* = 13) in the Gulf of Gabès; *N*d: number of debris; *N*t: number of turtles for which debris was found.

Marine debris type	***N*d**	***N*t**	**Occurrence (%)**
Soft plastics	6	4	30.77
Ropes	11	4	30.77
Wood	5	2	15.38
Fish hooks	3	3	23.08

Table 3. Frequency of occurrence of anthropogenic marine debris ingestion in *Coretta caretta* in the central Mediterranean Sea (CCL, Curved Carapace Length; *N*, number of individuals included in the study).

Locality	***N***	**CCL range (cm)**	**Occurrence (%)**	**References**
Western Mediterranean (Spain)	54	34.0–69.0	79.63	Tomás *et al.* 2001
Western Mediterranean (Valencia region, East Spain)	155	11.0–80.0	78.1	Domènech *et al.* 2019
Tyrrhenian Sea (Sardinia, Tuscany, Lazio Campania, Italy)	150	21.0–82.7	85	Matiddi *et al.* 2017
Tyrrhenian Sea (Gulf of Naples, Italy)	54	-	1.6	Bentivegna *et al.* 2003
Tyrrhenian Sea (Lazio and Campania regions, Italy)	61	22.0–81.8	91.5	Mariani *et al.* 2023
Adriatic Sea (Slovenia and Croatia)	54	25.0–79.2	35.2	Lazar and Gracan (2011)
Adriatic Sea (Abruzzo and Molise regions, Italy)	89	19.2–107.0	43.83	Mariani *et al.* 2023
Adriatic Sea (Gulf of Manfredonia, Italy)	76	32.3–81.7	35.1	Baldi *et al.* 2023
Central Mediterranean Sea (Lampedusa Island, Italy)	79	25.0–80.3	48.1	Casale *et al.* 2008
Central Mediterranean Sea (Lampedusa Island and Sicily, Italy)	567	18.2–82.0	35.4	Casale *et al.* 2016
Central Mediterranean Sea (Malta)	99	20.0–69.5	20.2	Gramentz (1988)
Central Mediterranean Sea (Gulf of Gabès, Tunisia)	132	28–77.5	9.85	Present Study
Eastern Mediterranean (North Cyprus)	135	-	42.7	Duncan *et al.* 2024

all anthropogenic materials were small and seemingly not problematic for the turtles examined. Plastic debris, specifically soft plastic and ropes were ingested by eight turtles across various seasons of the year (2 in autumn, 1 in winter, 1 in spring, and 4 in summer).

Diet Shifts

The results of the MANOVA showed that there was no difference in the loggerhead diet on body size and sex (Table 4). The relative significance index (%IRI) of some prey items varied seasonally, indicating that molluscs and arthropods were ingested more frequently in spring and summer, whereas tunicates and poriferans were ingested more frequently in fall and winter; fish consumption was only recorded in spring and summer (Figure 6).

Discussion

Dietary Composition

The analysis of digestive contents of *Caretta caretta* in the Gulf of Gabès revealed a diverse array of prey items, non-prey remains and debris, as detailed below:

Prey.—Forty-six prey items were discovered in the digestive tracts of 132 *C. caretta,* 20 of which were in 66% of the animals studied. This finding supports the loggerhead turtles' opportunistic and predatory feeding patterns already suggested in other regions in the Mediterranean Sea (Godley *et al.* 1997, Tomás *et al.* 2001, Bentivegna *et al.* 2003, Casale *et al.* 2008, Hochscheid *et al.* 2013). Additionally, based on the frequency and mass, benthic organisms such as porifera, arthropods, and molluscs seemed to be the preferred prey of loggerhead sea turtles in the Gulf of Gabès. According to Zander's classification (1982), these items are categorized as essential prey (porifera and arthropods) and main prey (molluscs). These findings align with studies from other Mediterranean regions, which demonstrates that *C. caretta* mostly feed on benthic molluscs and crustaceans (Godley *et al.* 1997, Casale *et al.* 2008, Lazar *et al.* 2011, Baldi *et al.* 2023, Mariani *et al.* 2023).

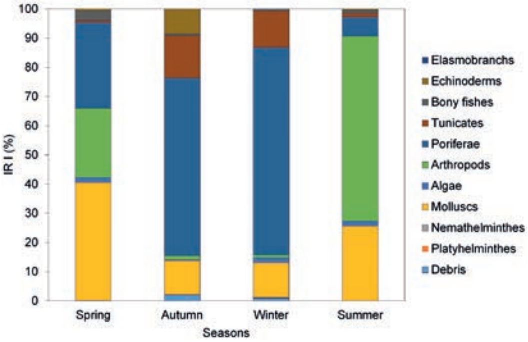

Figure 6. Seasonal variation in % IRI percentages of different items in *Caretta caretta* in the Gulf of Gabès; %IRI: Percentage index of relative importance.

The frequency of identifiable remains in the turtles' digestive tracts may be influenced not only by the types of prey consumed but also by the structural attributes of the prey that affect their retention within the turtles' digestive systems. Soft-bodied prey, such as cnidarians,

Table 4. Multivariate analysis of variance (MANOVA) table of Wilks' lambda. (*df,* degrees of freedom; W.L, value of Wilks lambda; *F*, approximate *F* value; H.*df*, hypothesis *df*; E.*df*, error *df;* Sig, significant.

Effect	*df*	W.L	F	H.*df*	E.*df*	Sig.
Size	1	0.85	1.85	10	103	0.06
Sex	1	0.79	1.28	20	206	0.19
Size * Sex	1	0.77	1.46	20	206	0.10

are less likely to be observed, whereas more robust prey, such as gastropods or crabs, are more likely to be detected. The abundance of various prey items found in the digestive tract of *C. caretta,* including the sea cucumber, *Holothuria polii* Delle Chiaje, 1824, the gastropod, *H. trunculus,* and the crab, *E. crenata,* aligns with their presence and distribution in the infralittoral and circalittoral bottoms of southern Tunisia (Bradaï 2000, Elhasni *et al.* 2010, El Lakhrach *et al.* 2012).

Sponges, *Chondrosia reniformis* and *Tethya* sp., were found in 12.1% and 7.69% of the cases, respectively. They were completely undigested in all instances, suggesting that *C. caretta* is incapable of digesting sponges, unlike hawksbill turtles, *Eretmochelys imbricate* (Linnaeus, 1766) (Bjorndal 1997). Possible explanations have been proposed for the ingestion of sponges: (1) mistaken prey identity (Steuer 1905, Acevedo *et al.* 1984); (2) sponges are a source of bacterial fauna or oligoelements (Laurent and Lescure 1994); and (3) accidental ingestion while targeting other prey (Casale *et al.* 2008). The significant weight of sponges in the dataset, largely due to two samples containing over 2 kg of *C. reniformis* each, inflated their IRI% value but may not accurately reflect their dietary importance at the population level. The substantial amount of *C. reniformis* supports the hypothesis of accidental ingestion of sponges. Notably, *C. reniformis* was the most frequently identified sponge and has been previously reported in the diet of trawl bycaught and in stranded turtles in the Mediterranean Sea (Casale *et al.* 2008, Palmer *et al.* 2021, Baldi *et al.* 2023).

Fish were the fourth most frequent taxon in the diet of *C. caretta* (18.7% *F*; 3.5% W). Apart from syngnathids, whose slow movement makes them easier prey (Brongersma 1972, Burke *et al.* 1993, Kleiber *et al.* 2010), other prey like cephalopods are generally not considered part of the loggerhead's natural diet (Plotkin *et al.* 1993, Laurent and Lescure 1994). Loggerhead turtles have limited capability to capture fast-moving and highly maneuverable prey (Plotkin *et al.* 1993, Tomás *et al.* 2001); however, maneuverable species are frequently caught by fishing gears, particularly trawlers, and become part of their discard (Sánchez *et al.* 2007), making them more easily accessible to turtles.

Two possible origins could be attributed to the fish sampled in our study. The first hypothesis proposes that turtles might opportunistically consume fish when caught and placed on the deck of fishing boats (Laurent and Lescure 1994, Seney 2003). The second and more plausible hypothesis suggests that the fish might be discarded by trawlers and later scavenged from the seafloor by turtles (Tomás *et al.* 2001, Seney and Musick 2007, Casale *et al.* 2008, Benhardouze *et al.* 2021, Palmer *et al.* 2021). This argument is supported by the presence of *Mullus* sp. and *D. vulgaris* in the turtle's diet, two species commonly targeted by trawlers in our study area (Jarboui *et al.* 2005, El Lakhrach *et al.* 2019).

Similarly, Cardona *et al.* (2024) suggested that the decline in fish consumption by *C. caretta* along the Spanish Mediterranean coast could be linked to a reduction in the fishing fleet in that area. In the Mediterranean, trawlers target multiple species and produce substantial discards (Tsagarakis *et al.* 2014), making it available for turtle consumption (Mariani *et al.* 2023). This has important conservation implications, since *C. caretta* are also scavengers and may be attracted to areas with intense fishing activity due to the availability of discards, which increases their probability of incidental capture (Baldi *et al.* 2023).

The opportunistic carnivorous diet of *C. caretta* is supported by the presence of tunicates such as *Pyura dura* (Heller, 1877) and *Microcosmus* sp. reported in this study. To date, tunicates are rarely reported in the diet of loggerhead turtles. Pelagic tunicates, such as *Pyrosoma atlanticum* (Péron, 1804), have only been documented in early pelagic juveniles from the western Mediterranean Sea and the North Atlantic (Brongersma 1972, Van Nierop and Den

Hartog 1984, Tomás *et al.* 2001). Among benthic tunicates, the only species previously identified in the diet of *C. caretta* was *Molgula manhattensis* (De Kay, 1843) found in Chesapeake Bay, Virginia, USA (Seney and Musick 2007).

Echinoderms were found only seven times in our study (%*F* = 7.7% and *N*% = 4.6), whereas in the research conducted by Casale *et al.* (2008), they were part of the diet of over 25% of loggerhead turtles. This discrepancy is probably attributed to differences in substrate composition. Frequenting trawled areas could expose turtles to habitats with diminished biodiversity, as repeated trawling activity often leads to the decline of some taxa (Handley *et al.* 2014, Baldi *et al.* 2023) and the replacement of native species by more opportunistic or scavenging species.

Algae and plants were part of the diet of 25 turtles (%*F* = 27.5). The presence of algae in the digestive contents of *C. caretta* has been reported in the Mediterranean (Basso and Cocco 1986, Tomás *et al.* 2001, Casale *el al.* 2008) and the Atlantic (Acevedo *et al.* 1984, Frick *et al.* 2009), where turtles likely ingest algae incidentally among the floating debris. In this study, the algae recovered could also originate from the seafloor of the Gulf of Gabès, where these species are commonly found (Zaouali 1993, Hattour and Ben Mustapha 2013). Notably, these algae were also undigested, corroborating the strictly carnivorous behavior of the studied species. Unable to digest plant matter, the turtles probably ingested the algae accidentally while preying on animal species (Casale *et al.* 2008). However, their presence suggests that loggerhead turtles might use shallow marine areas with ample light penetration, particularly regions abundant in *Posidonia* and *Cymodocea* seagrass beds. These shallow benthic environments, characterized by extensive seagrass beds, serve as habitats for a highly diverse set of species and are commonly utilized by loggerheads for foraging (Godley *et al.* 1997, Houghton *et al.* 2000, Casale *et al.* 2008, Lazar *et al.* 2011, Patel *et al.* 2016).

Three non-indigenous species previously reported in the Gulf of Gabès were observed in the diet of the sampled turtles: the bivalve, *Pinctada radiata,* and the crab, *E. crenata,* both originating from the Indo-Pacific, and the West Atlantic crab, *Libinia dubia* (Ben Amor *et al.* 2016). Since the sampling period occurred before 2014, when the blue crab, *Portunus segnis* (Forskål, 1775), was first report in Tunisian waters (Rabaoui *et al.* 2015), this species was not reported as part of the diet of the necropsied turtles. In recent years, the species has been reported in the diet of these turtles (Bradaï *et al.* 2017), suggesting that loggerhead sea turtles can serve as a tool for monitoring potential new invasive species.

Non-prey remains.—Barnacles of the species *Balanus perforatus* Bruguière, 1789 were identified among the remains. Sea turtles epibionts, such as amphipods and cirripeds, have been previously documented in the diet *of C. caretta* sampled in the Atlantic (Frick *et al.* 2009). These organisms are likely ingested while attached to anthropogenic debris floating on the surface (Frick *et al.* 2009). As one of the most common fouling organisms (Christie and Dalley 1987), barnacles can be consumed by sea turtles indirectly through their association with organisms such as crabs (Karaa *et al.* 2018) or pearl oysters (Abdelsalam and Elebiary 2023).

Non-organic matter (Debris).—Anthropogenic debris was present in 9.85% of the turtles. This proportion is higher than reported for *C. caretta* sampled in the Gulf of Naples between 1991 and 2001 (only 1.6% of 40 turtles examined) but lower than values recorded in other regions of the central Mediterranean. These differences suggest a spatial and temporal variability in the distribution of debris or its consumption by turtles (Table 3). Several factors may influence these findings, including turtle recovery method, the age of the turtles, and the approach used to characterize marine debris ingestion. Differences in the frequency of debris consumption may provide insight about the pollution level in those areas. In our study, debris were more frequently observed in the summer, coinciding with increased turtle activity at the water's surface. During this period, turtles often need to surface for air, making them more vulnerable to capture and recapture. This can result in stress and lead to buoyancy issues, prompting them to accidently ingest floating debris while foraging on surface-associated organisms.

In addition, three turtles ingested natural debris, such as sand, likely while foraging on the seabed. As part of their feeding behaviour, *C. caretta* often search the ocean floor for food, which can result in incidental sand ingestion (Preen 1996). All of the anthropogenic debris, except for fishhooks, consisted of small fragments. This observation, combined with the absence of apparent pathologies linked to debris ingestion, suggest that it was not a direct cause of death. However, the long-term effects of plastic consumption, especially from repeated intake over time, remain poorly understood. Non-lethal ingestion of debris has been documented in several studies (e.g., Tomás *et al.* 2002, Revelles *et al.* 2007, Casale *et al.* 2008, 2016, Lazar and Gracan 2011). Plastic debris retained in the digestive tract can increase the risk of internal injuries and cause sub-lethal effects such as reduced stomach capacity, inappetence, buoyancy issues, and chemical contamination. Over time, these consequences can harm turtles' health, reducing their fitness and potentially leading to death (Nelms *et al.* 2016).

Regional Variability in Diet

Loggerhead turtles' diets in the Mediterranean vary according to feeding habitats and availability of prey species. In the Balearic Islands, they primarily feed on the Mediterranean jellyfish, *Cotylorhiza tuberculate* (Macri, 1778) (Revelles *et al.* 2007). In the central Mediterranean, hermit crabs serve as a major food source (Casale *et al.* 2008). Along the Greek coast, they target Mediterranean mussels (*Mytilus galloprovincialis*, Lamarck, 1819) (Houghton *et al.* 2000), while in the Adriatic Sea, they feed on European clams (*Corbula gibba* Olivi, 1792), and crabs (*Liocarcinus* sp.; and *Goneplax rhomboids* Linnaeus, 1758) (Lazar *et al.* 2011, Mariani *et al.* 2023).

In the Gulf of Gabès, loggerheads predominantly consume the Blunt-spined euryplacid crab (*E. crenata*), the cuttlefish (*S. officinalis*), the Banded murex (*H. trunculus*), and the Spot-tail Mantis Shrimp (*S. mantis*). This study expanded the data on the phyla and species preyed on by the loggerhead sea turtles in the Tunisian Plateau (Central Mediterranean Sea). It also emphasizes the use of dietary analysis, which offers insights into marine biodiversity and the distribution of non-native species in the area.

Relationship Between Diet and Used Marine Habitats

Caretta caretta in the Gulf of Gabès primarily feed on prey from benthic neritic zones, as their dietary data revealed. They commonly consumed the crab, *E. crenata,* the cephalopod, *S. officinalis,* and the gastropod, *H. trunculus,* which inhabit the sublittoral zone at depths of 1030 meters (Zaouali 1993, Elhasni *et al.* 2010, El Lakhrach *et al.* 2012). Additionally, these turtles also exhibited pelagic feeding behavior. They ingested floating debris, such as wood and plastic, and pelagic prey like Clupeidae (*S. aurita*). The alternate use between benthic and pelagic habitats has been supported by numerous studies that documented the bycatch of juvenile turtles by surface and bottom longlines in the study area (Jribi *et al.* 2008, Echwikhi *et al.* 2011). The natural characteristics of Gulf of Gabès, including its shallow depth (less than 50 m) and wide continental shelf, enable loggerhead turtles to forage across the entire water column. This habitat structure allows turtles to alternate between pelagic and benthic zones with minimal energy expenditure. Our findings support previous research showing that both juvenile and adult loggerhead turtles adopt an opportunistic amphi-habitat feeding strategy. As they mature, however, they increasingly prefer benthic prey (Casale *et al.* 2004, 2007a,b).

Diet Shifts

Diet and turtle size.—Turtle size does not appear to affect food preferences. Studies on *C. caretta* from the western Mediterranean Sea, the Tyrrhenian Sea and the Adriatic Sea show similar feeding behavior regardless of size (Haywood *et al.* 2020, Baldi *et al.* 2023, Mariani *et al.* 2023). The absence of evidence for a size-related habitat shift may be explained by an intermediate neritic transition phase for juvenile turtles (Casale *et al.* 2018, Haywood *et al.* 2020). Conversely, other authors suggest that the number and diversity of prey species (mostly benthic) increased with turtle size (Tomás *et al.* 2001, Youngkin 2001). In a study conducted in thesoutheast US, researchers found that adult turtles consume more gastropods, while subadults consume more fish, indicating that the size of the turtles might influence the proportions of certain prey (Molter *et al.* 2022). Given that most of the turtles sampled in the current study were primarily PPJ, further investigation and a larger sample size are needed to better understand the impact of turtle size on diet in the Gulf of Gabès.

Seasonal changes in diet.—The seasonal variation in the relative prey importance index (%IRI) reveals shifts in the types of prey ingested across different seasons. Molluscs, arthropods, algae, tunicates, and porifera were consumed throughout the year. Molluscs and arthropods were more frequently consumed in spring and summer, whereas tunicates and poriferans were more frequently consumed in fall and winter. This seasonal shift in prey types may relate to reduced activity in *C. caretta* during colder seasons (Hochscheid *et al.* 2005). Additionally, opportunistic feeding behavior may explain the consumption of teleosts (fish) during the warm months.

Even during the colder months, loggerhead turtles in the Gulf of Gabès continue feeding on benthic organisms such as sponges, gastropods, and bivalves. Similar behavior has been observed in other locations in the Mediterranean (Hochscheid *et al.* 2005), in South Africa (Hughes 1974), and in the United States, particularly the Gulf of Mexico (Plotkin *et al.* 1993). These findings highlight the adaptability and resilience of *C. caretta,* enabling them to sustain feeding habits year-round, even during the winter. By adopting a “sit-and-wait” overwintering strategy with infrequent surfacing, these chelonians sufficiently reduce the metabolic costs of overwintering (Hochscheid *et al.* 2005). Satellite tracking data from the Mediterranean demonstrated that these turtles are not obligate hibernators (Hochscheid *et al.* 2007). Wintering strategies differ within the Mediterranean basin, as warmer temperatures in the eastern region may allow turtles to remain active, continue feeding, and take fewer rest periods during winter (Hochscheid *et al.* 2007).

Conclusion

This study highlights the value of diet analysis in revealing the feeding ecology and habitat use of loggerhead turtles in the Gulf of Gabès. The majority of *Caretta caretta* in this region are post-pelagic juveniles and exhibit opportunistic feeding behaviour, consuming trophic resources from both pelagic and benthic environments. Their diet changes with the seasons, and they remain active during the winter without hibernating.

The Gulf of Gabès, which has the most productive marine habitats in Tunisian waters, is likely to be one of the most important areas for loggerhead turtles in the Mediterranean (Casale and Margaritoulis 2010). Satellite telemetry and flipper tagging data suggest that the region is home to 28 to 44.4% of the females nesting in western Greece (Patel *et al.* 2015). Efforts to protect and manage this area, ensuring the availability of diverse and healthy trophic resources in both pelagic and benthic zones, could be pivotal for supporting populations of *C. caretta* and promoting their survival in Tunisian waters. The importance of the Tunisian plateau for loggerhead turtles in the Mediterranean highlights the need for sustained, long-term monitoring efforts to assess the conservation status of these chelonian populations in the region (Karaa *et al.* 2016 a,b). Future efforts should prioritize analyzing gastrointestinal contents as an ecological indicator and biodiversity markers to enhance our understanding and protection of marine turtles in the region.

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Movement and habitat use of female subadults of *Podocnemis expansa* (Testudines: Podocnemididae) in northern Brazil

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Abstract

Movement and habitat use of female subadults of *Podocnemis expansa* (Testudines: Podocnemididae) in northern Brazil. The Giant South American River Turtle, *Podocnemis expansa,* is recognized for its social interactions across all sexes and age/size groups. Its movement patterns, especially those of subadults, are poorly understood. Movement is a crucial factor for the effective conservation and management of wildlife. From October 2010 to September 2011, using VHF radio-tracking methods, we studied the movements of eight subadult females in the Trombetas River Biological Reserve (REBIO do Rio Trombetas) in Pará State, Brazil. We observed subadult females utilizing lake-channel systems near the Trombetas River with their movement rates positively influenced by their size and negatively by the water level of the river. Individuals remained in the flooded forest (igapó) during flooding, likely feeding on fruits and seeds. During the nesting season, we observed interactions between adult and subadult females near the nesting sandbanks. The proximity of subadult and adult Giant South American River Turtles suggests that social interactions, such as learning reproductive and nesting behaviors, could be vital for the movement of subadult females, going beyond simple resource availability.

Keywords: Amazonian turtles, Home range size, Radio-tracking, Social behavior, Water level.

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Resumo

Movimentação e uso do habitat de fêmeas subadultas de *Podocnemis expansa* (Testudines: Podocnemididae) no norte do Brasil. A tartaruga da Amazônia, *Podocnemis expansa*, é reconhecida pelas suas interações sociais em todos os sexos e grupos de idade/tamanho. No entanto, seus padrões de movimento, especialmente os dos subadultos, são pouco conhecidos. O movimento é um fator crucial para a conservação e gestão eficaz da vida selvagem. De outubro de 2010 a setembro de 2011, usando métodos de monitoramento de rádio VHF, estudamos os movimentos de oito fêmeas subadultas na Reserva Biológica do Rio Trombetas (REBIO do Rio Trombetas), no Estado do Pará, Brasil. Observamos fêmeas subadultas utilizando sistemas de lagos e canais próximos ao rio Trombetas, com suas taxas de movimentação influenciadas positivamente pelo seu tamanho e negativamente pelo nível da água do rio. Os indivíduos permaneceram na floresta inundada (igapó) durante a cheia, provavelmente se alimentando de frutos e sementes. Durante a época de nidificação, observamos interações entre fêmeas adultas e subadultas próximas aos bancos de areia de nidificação. A proximidade entre subadultos e adultos sugere que interações sociais, como o aprendizado de comportamentos reprodutivos e de nidificação, podem ser vitais para o deslocamento de fêmeas subadultas, indo além da simples disponibilidade de recursos.

Palavras-chave: Comportamento Social, Nível da água, Radiotelemetria, Tamanho da área de vida, Tartaruga-da-amazônia.

Introduction

Turtles in the Family Podocnemididae Cope, 1868 exhibit movement patterns and habitat use that are closely linked to changes in water levels that result from the annual flooding of Amazonian rivers (Fachín-Téran *et al.* 2006, Leite 2010, De la Ossa and Vogt 2011, Perrone *et al.* 2014, Leão *et al*. 2019). Research on chelonian movement in Amazonia remains limited. Understanding animal movements and interactions, as well as how environmental processes shape species distributions, helps develop targeted conservation strategies, manage natural resources sustainably, and predict how species might respond to environmental changes such as habitat loss, climate change, and human disturbances (Famelli 2013, Hussey *et al.* 2015, Cueva *et al.* 2018). Radio-tracking studies are essential for understanding life history and determining ecological patterns in wildlife populations (Magnusson *et al.* 1997, Hussey *et al.* 2015, Famelli *et al.* 2016, Leão *et al.* 2019). Over the past decade, radio-tracking studies on freshwater turtles have increased substantially, generating valuable insights into movement patterns, habitat use, seasonal migrations, linear displacements, and comparisons between methods for measuring home range sizes (Ribeiro *et al.* 2024). Despite this progress, significant gaps persist in our knowledge of most species in the Neotropical region, which is especially concerning given the region's high endemism and exceptional diversity of freshwater turtles (Ribeiro *et al.* 2024). The Giant Amazonian River Turtle, *Podocnemis expansa* (Schweigger, 1812), is the largest South American freshwater chelonian found in the basins of the Orinoco and Essequibo rivers and the drainages of the Amazon River, spanning Colombia, Venezuela, Guyana, northwestern Peru, eastern Ecuador, northern Bolivia, and northern and central-western Brazil (Rueda-Almonacid *et al.* 2007, Ferrara *et al.* 2017, Forero-Medina *et al.* 2019). The species also migrates over hundreds of kilometers (Pezzuti *et al.* 2004, Carneiro and Pezzuti 2015), and, as the largest member of Podocnemididae, it is expected to show the largest home ranges among the species within the taxonomic group. Therefore, studies on the movements of *P. expansa* will provide valuable insights into the biology of Amazonian chelonians.

Although individuals of *P. expansa* with carapace lengths exceeding 1 meter were once commonly observed in Amazonian rivers (Ernst and Barbour 1989), sightings of such large specimens have become rare. This decline is primarily attributed to the historical loss of adult females during nesting activities, as evidenced by the reduction in the number of hatchlings released annually in certain areas inhabited by *P. expansa,* despite decades of nesting site protection (Cantarelli 2006, Vogt 2008, Forero-Medina *et al.* 2019, Lacava *et al*. 2024). Females of the species reach sexual maturity around 50 cm (Alho and Pádua 1982a, von Hildebrand *et al.* 1997), whereas males mature around a carapace length of 30 cm (Portelinha *et al.* 2013, 2014). The species is fully aquatic, with large groups of females observed aggregating in the water during the dry season, when sandy nesting beaches emerge, and leaving the water as a group in what is known as arribada nesting (Alho and Pádua 1982a, b, von Hildebrand *et al.* 1997, Hernandez *et al.* 1998, Rueda-Almonacid *et al.* 2007, Simoncini *et al.* 2022). Among freshwater chelonians, *P. expansa* is undoubtedly one of the most social turtle species worldwide (Ferrara *et al.* 2013, 2014). This species exhibits a complex vocal repertoire and audible communication across all age categories, coordinating mass nesting, nights without nesting, synchronized emergence of hatchlings from nests, and post-hatchling parental care (Ferrara *et al.* 2013, 2014, Jorgewich-Cohen *et al.* 2024).

Extensive exploitation of *P. expansa* since the late 18th century has driven significant population declines. This species has been severely targeted by humans for its high nutritional-value protein and subjected to illegal trade in clandestine markets (Bates 1863, Smith 1979, Rebelo 2002, Pezzuti *et al*. 2004, Cantarelli 2006, Rueda-Almonacid *et al*. 2007, Forero-Medina *et al*. 2019). As a result of ongoing threats, *P. expansa* has recently been reassessed and is presently in the process of being categorized as Endangered by the International Union for Conservation of Nature Red List of Threatened Species (IUCN; C. R. Ferrara *et al.* pers. obs.). In the Trombetas River Biological Reserve, Pará, Brazil, human impact has caused the number of nesting females to drop from over 8000 in 1976 to just 850 in 1989 and further declining to fewer than 350 females since 2008 (Ferrara *et al*. 2010, Forero-Medina *et al*. 2019). In this context, understanding the movement patterns and home range needs of *P. expansa* in the Trombetas River is essential, especially as this region once harbored one of Brazil's largest populations of *P. expansa,* with significant decreases over the years (Forero-Medina *et al*. 2019). Examination of movement patterns across various size classes and life stages is critical to informing effective, targeted management strategies.

Concerns about significant declines in *P. expansa* populations have driven numerous research and conservation approaches in Amazonia, compelling scientists, NGOs, and community programs to implement protective strategies aimed at understanding and preventing the underlying causes, ecological impacts, and potential conservation measures to protect and restore this endangered species (Forero-Medina *et al*. 2019, Andrade *et al*. 2022). Despite being extensively studied, conservation efforts for Amazonian chelonians primarily focus on safeguarding nesting sites to protect females and the early life stages (eggs and hatchlings) (Cantarelli 2006, Miorando *et al*. 2013, Cantarelli *et al*. 2014). A significant research gap exists concerning other life stages of *P. expansa,* particularly subadults, which are crucial in the population's transition to adulthood (Pritchard and Trebbau 1984, Cantarelli 2006). There is evidence, however, that prioritizing the survival of juveniles and adults over fecundity is the most effective conservation strategy for reversing population decline (Mogollones *et al*. 2010). Given the scarcity of data on ontogenetic shifts of the movement, activity, home range sizes, and habitat of *P. expansa,* this study aimed to elucidate these aspects by radio-tracking subadult females throughout the hydrological cycle in the Biological Reserve of Trombetas River, Pará. This study (a) evaluates the effect of the Trombetas River level on the average monthly distance traveled by individuals; (b) estimates their areas of use or home range sizes; (c) assesses the degree of overlap and social interactions in areas of use; (d) and characterizes habitat use across dry-flood periods.

Materials and Methods

Study Area

The Trombetas River Biological Reserve (REBIO Trombetas: 00°39'–01°29' S, 56°17' –57°03' W) covers approximately 385,000 ha, and is situated in the municipality of Oriximiná, Pará state, Brazil. This area encompasses a significant portion of the northeastern region of Pará and is nestled within the Amazon Basin in the interfluve of the Trombetas and Acapu rivers (Figure 1). The Trombetas River is a clear-water river that extends 800 km toward the Venezuelan border and is the second largest tributary on the left margin of the Amazon River (IBAMA and STCP 2004). Many species of *Podocnemis* utilize the large sandbanks in the region for nesting during the dry season. These large nesting sites provided critical habitat for the reproduction of turtles; thus, their presence was instrumental in the creation of the reserve in 1979 (IBAMA 1989). The biologically diverse area within the REBIO do Rio Trombetas encompasses a rich array of aquatic habitats, including numerous lakes and channels that flow into the rivers (IBAMA and STCP 2004).

The REBIO do Rio Trombetas represents a unique and ecologically significant area within the Amazon region, offering valuable insights into the intricate relationships between hydrology, biodiversity, and human communities (IBAMA and STCP 2004, Eisemberg *et al*. 2016). Traditional communities, including riverine and quilombola communities, occur in the reserve and use chelonians for their subsistence. The area faces threats from illegal harvesting and egg collection, posing challenges to the conservation of turtles, especially related to enforcement (Eisemberg *et al*. 2016).

Data were collected along a stretch of approximately 20 km of the Trombetas River, covering an area of 70 km^2 in the central region of the river. This area includes the Jacaré and Leonardo lakes, as well as nearby systems such as the Farias and Abelardo system lakes (Figure 1). Additionally, the study site also comprises seasonally flooded forest areas during the high-water period.

Trombetas River Cycle

The Amazon Basin can be divided into four hydrological seasons related to the rising and falling of the level of the rivers. In the REBIO do Rio Trombetas, the water level is at its lowest in October and November. Rain begins in late November, and the rivers begin to rise, flooding the forests and reaching its peak in March and April. As the rain diminishes in intensity, the water gradually recedes until the forest is no longer flooded by late August, when the turtles begin congregating to migrate to the nesting beaches (Alho and Pádua 1982a, Leão *et al*. 2019). The seasons in the Trombetas River include four stages: (a) Receding (falling water level), which generally happens from July to October; (b) Dry (lowest water level), October to December; (c) Flooding (water level rising gradually), December to April; and (d) Full (highest water level), April to June.

The water level of the Trombetas River was expressed in meters, representing the difference in water column level relative to the average level of the Atlantic Ocean (*cota*) in Brazil. Elevation values were obtained from the "Base Tabuleiro" river gauge station provided by the National Water Agency (ANA). Based on daily elevation data from October 2010 to September 2011, four phases of the hydrological cycle were

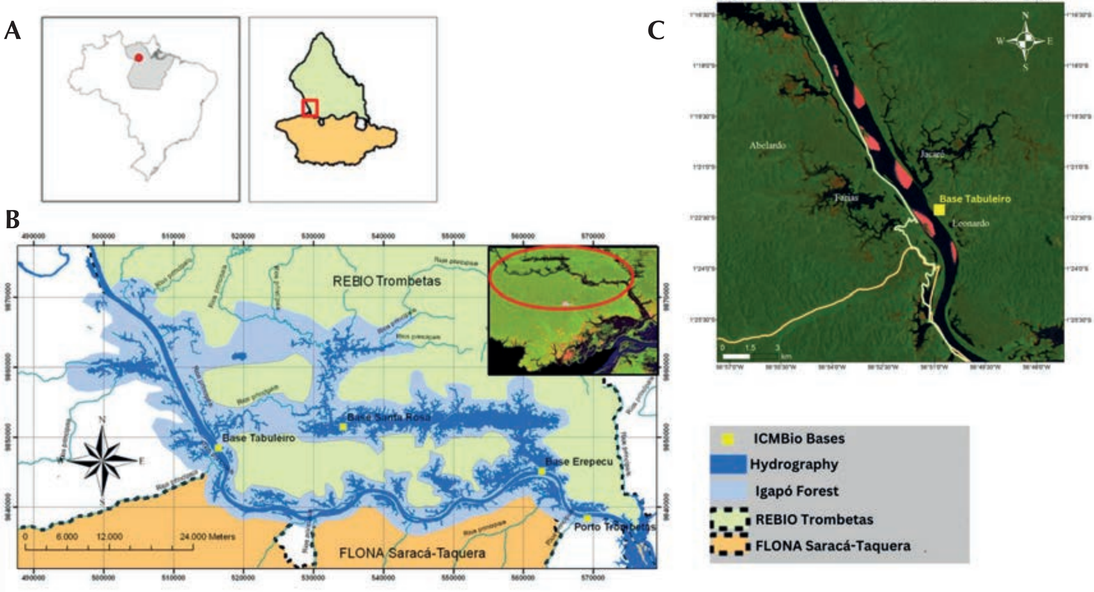

Figure 1. (**A**) Geographical boundaries of the Trombetas River Biological Reserve (REBIO do Rio Trombetas), highlighting the border with the Saracá-Taquera National Forest (FLONA Saracá-Taquera), Pará state, northern Brazil. The satellite image in the upper right corner (**B**) shows a section of the Trombetas River within the limits of the REBIO and FLONA, marked by the red circle, extending to the mouth of the Trombetas River on the left bank of the Amazon River. (**C**) Sampling area, highlighting Jacaré, Leonardo, Abelardo, and Farias lakes. Along the main course of the river are sandbanks (marked in pink) that emerge during the dry season.

established for the entire sampling period (Figure 2; Bittencourt and Amadio 2007).

Capture, Marking, and Morphometry

Between October and December 2010, and from April to June 2011, we captured a total of 14 turtles using trammel nets and active search (Enríquez-Mercado *et al*. 2024). Individuals were marked by notching marginal scutes of the carapace, following established mark-recapture protocols (Cagle 1939). We obtained the straight-line carapace length (SLC) and the body mass (BM) of each individual using caliper and spring scale dynamometers, respectively (Enríquez-Mercado *et al*. 2024). Sex was determined by observing the opening angle of the anal plate and the size of the tail (larger in males) (Vogt 2008).

Radio-tracking: Fitting and Operation

We selected 12 subadult females to be equipped with the VHF radio-transmitters (AVM Instrument Co. Model MP2, California, USA). This selection was based on criteria including CL measurements lower than the minimum maturation size established in the literature (50 cm for females; Alho and Pádua 1982a, von Hildebrand *et al*. 1997) and compatibility of individual mass with that of the radio-transmitter, ensuring it did not exceed 10% of the individual's BM (Jacob and Rudran 2006). The radio-transmitter device operates on a single frequency of radio waves between 164 and 165 MHz. We affixed the devices to a turtle's carapace using Tubolit® underwater glue, positioned on the 2nd and 3rd vertebral shields. After installation,

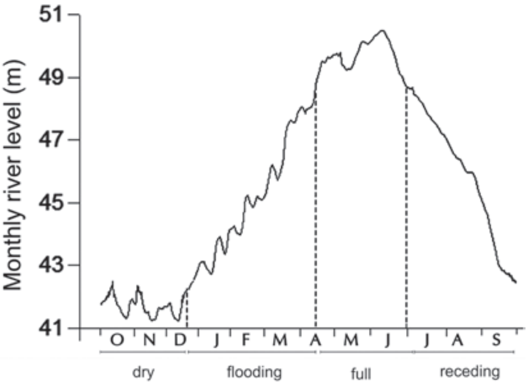

Figure 2. Monthly level of the Trombetas River (m) between October 2010 and September 2011 at Trombetas River Biological Reserve, Pará state, northern Brazil.

turtles were kept in empty fiberglass tanks for 24 hours to allow the glue to completely adhere to the carapace before being released at the capture sites. The carapace length (CL) of turtles monitored ranged from 20 to 40.2 cm (28.3 ± 2.4) and body mass (BM) from 0.9 to 6.6 kg (2.9 ± 0.7). To capture the radio-transmitter's signal we used a three-element Yagi model unidirectional antenna connected to a powered radio receiver (ATS Model R2000 Scientific Receiver) via a two-meter-long coaxial cable. An external 12V battery powered the receiver. To minimize external noise interference, we utilized a headset during tracking. Additionally, a wooden stick approximately 1.7 meters in length was employed to raise the antenna, thereby increasing the signal reception range in open areas (Jacob and Rudran 2006). We lost the signal from four subadult females the day after their release and were unable to locate them for the entire study period. Therefore, only eight subadult females were monitored.

The digital memory of the radio receiver facilitated the storage of transmitter frequencies. We used the receiver's scanner function to perform periodic scans of these stored frequencies at eight-second intervals, ensuring each radio-tagged turtle had an equal chance of being detected. After we recorded the location, we removed the corresponding frequency from the receiver's memory. Given the limited visibility caused by the dark water color in lakes and flooded forests, we directed the antenna toward the area with the strongest signal, disconnected the coaxial cable and headphones, and confirmed the location only upon hearing the distinct signal emitted by the receiver (Jacob and Rudran 2006).

The radio-tracking data were collected four to six times per week from October 2010 to September 2011, encompassing a complete hydrological cycle. Each turtle was tracked once daily to minimize the risk of losing its location, with a minimum 24-hour interval between consecutive readings to mitigate temporal autocorrelation effects and ensure statistical independence (Swihart and Slade 1985). Each location record included the date, time, geographic coordinates obtained using a Garmin GPS device (maximum satellite error of eight meters), and details regarding the environmental characteristics of the turtle's habitat.

Tracking operations were carried out from an aluminum boat equipped with a 15-hp engine, with one person navigating and another handling the tracking tasks. The boat's speed during searches was kept below 15 km/h, aligning with the natural flow of the water bodies. In flooded forest areas (igapó) during the flood season, we used a wooden boat and oars.

Habitat Use

To assess environmental variation in habitat use we conducted sampling across two distinct periods: the first in October 2010 (during the dry season) and then in the flood season (June 2011). Characterization of the environment was primarily based on observations made during the dry season, which facilitated the identification of submerged features such as surrounding vegetation types, substrate composition (e.g., silt, sand, clay, litter), and water flow speed categorized as either lentic or lotic (Table 1). Additional details observed throughout the

Table 1. Characterization of the aquatic environments identified between October 2010 and September 2011 in the sampling area at Trombetas River Biological Reserve, Pará state, northern Brazil.

Category	Habitat characteristics
Freshwater lake (FL)	Permanent lentic environment and marginal vegetation; occurrence of "pools" (places with high depths, greater than 8.0 meters, even during the dry season). During floods (December to January), banks of wild rice (*Oryza glumaepatula*) form on the surface of the water, close to the banks. Muddy substrate covered with decomposing plant material (tree trunks, branches, leaves).
River Chanel (RC)	Lotic environment, in the main course of the river, with depths that varied between 6.0 and 19 m in the sampled locations, with the presence of pools and absence of vegetation.
Open *cuiarana* flooded forest (OCFF)	Lentic environments with flooded vegetation, where the plant species *Eschweilera* sp. predominates (*cuiarana*). The trees are restricted to the low topographic levels of the land, subject to periods of six to eight months of flooding, and are spaced apart, allowing intense light to enter. During floods, banks of wild rice (*O. glumaepatula*) can form next to "*cuiarana*" trees. Substrate formed by silt and sand.
Open flooded forest mixed (OFFM)	Lentic environment of flooded forest, with a predominance of *Symmeria paniculata* (*carauaçu*) shrubs and trees measuring between 7.0 and 15 meters, spaced apart, which include *Macrolobium* sp. (*arapari*); *Eschweilera* sp. (*cuiarana*). The topographic elevation of the land is the lowest, subjecting plant species to long periods of flooding (up to eight months). The occurrence of aquatic macrophytes is common. The substrate is made up of silt, litter, and sand.
Flooded forest (FF)	Lentic environment of flooded forest. Formed by medium to large trees (more than 15 m) and with little light passing between them. Trees such as *Acosmium nitens* (*itaubarana*), *Ormosia* sp. (*tento*), *Campsiandra* sp. (*acapurana*), *Astrocaryum jauari* (jauari), *Euterpe precatoria* (*açaí*) were common, among others.
Cano (C)	Lotic watercourse that connects a lake to the main channel of the river at flooding and beginning of ebb; at the height of the drought, the lake becomes accessible only by land. Water flows in the river–lake direction during the flood and the reverse occurs during the ebb. Presence of bushy tangles of various plant species. Silty substrate–clayey.
Vegetated ravine (VR)	The marginal area of the river with a predominance of clay soil and vertical gradient with the presence of shrub to tree vegetation, which can be seasonally flooded in places with lower topography.
Bay Beach (BB)	Lentic, shallow (up to 5 m), bay-shaped environment on the banks of sandy beaches (large sandy soil outcrops in the main course of the river). This environment is locally known as a "*ressaca*."

tracking period were incorporated into the environmental descriptions. Analysis of the collected data enabled the identification of eight primary environmental categories in this study, drawing upon the classification systems outlined by Leite (2010) and Martin *et al*. (2004), with some modifications.

Distances Traveled

We estimated the individual monthly distance (IMD) by calculating the sum of each individual's consecutive journeys per month, excluding monthly journeys of fewer than five tracking days. The average monthly distance

(AMD) traveled was estimated by summing the IMD of each individual and dividing it by the number of individuals monitored during the period. The total distance traveled (DT) by the turtles was the cumulative sum of the respective distances covered monthly during monitoring.

Home Range

We estimated the linear home range (LHR), represented by the straight-line or the maximum distance between the two most distant fixes, considering these movements as unidirectional (Morales-Verdeja and Vogt 1997, Fachín-Terán *et al.* 2006, Bernhard 2010, Freeman *et al*. 2018). We used the conventional 95% Minimum Convex Polygon ($MCP^{95\%}$) and the 95% Kernel Density Estimator ($KDE^{95\%}$) methods to estimate the size of the home range of the tracked turtles. The KDEs smoothing parameter (h) was determined using the least squares cross-validation method. Given the high autocorrelation in tracking data, we also employed the Autocorrelated KDE (AKDE; Fleming *et al*. 2015) to reduce the uncertainty associated with such data (Horne *et al*. 2007, 2019, Crane *et al*. 2021). For AKDE, we used UD2 (OUF) in the “ctmm” in R which considers the correlated positions and correlated velocities in the fixes. We also estimated the $MCP^{95\%}$ and $KDE^{95\%}$ of four adult females observed sharing the same area. These large females were equipped with VHF radio-transmitters (model MP2, AVM Instrument Company Ltd.) with a frequency of 165 MHz, during simultaneous research in the same area. Three of them were monitored during a study on underwater communication and parental care between adult females and hatchlings (Ferrara *et al*. 2013), and another one was part of a pilot project of the Richard Vogt lab (V. Bernardes, pers. comm., September 2011). Due to the limited number of relocations and the small sample size of adult individuals, these data are presented solely as exploratory and serve to provide insights into the overlap between adults and subadults.

Overlapping

We assessed spatial overlap among the monitored individuals by calculating the percentage of home range intersection. This analysis was accomplished using the $KDE^{95\%}$ method implemented in the ‘adehabitatHR’ package in R (Calenge 2006), which identifies centers of activity that are potentially associated with ecologically significant areas for chelonians, such as mating or nesting sites (Leão *et al.* 2019). This approach provides a comprehensive assessment of home range overlap and the potential frequency of spatial interactions among individuals (Famelli *et al*. 2025).

Data Analysis

We performed all analyses in the program R 4.3.0 (R Development Core Team 2023). The home range areas were saved either as raster or shapefiles and transferred to the ArcGIS program, version 10.5 (ESRI 2016). The comparison between home range estimators was assessed using ANOVA. We conducted a linear regression analysis to evaluate possible relationships between river monthly elevation (predictor variable) and distance traveled (response variables). We also performed linear regressions to examine the relationship among the home range sizes (response variable) to the body size (CL and BM; predictor variables), followed by ANOVA. The home range sizes of adults and subadults was compared using a Mann-Witnney *U* test. A significance level of ≤ 0.05 was adopted for all analyses.

Results

Habitat Use

We located the turtles equipped with radio-transmitters 535 times (an average of 66.9 locations per individual) between October 2010 and September 2011. Throughout the 11-month data collection, the chelonians were observed utilizing three lakes (Farias, Jacaré, Abelardo) and the Trombetas River, encompassing two micro basins situated on opposite banks of the river. The monitored turtles showed distinct variation in habitat use across different stages of the hydrological cycle. During the rising water phase, they relocated to the igapós. In contrast, during the low water phase, they predominantly utilized lakes and river habitats, favoring backwaters over the main river channel. As the igapó flooded due to lateral overflow from the Trombetas River, the turtles migrated from the river and lakes. During the low-level phase, when most igapó habitats became dry, the chelonians relocated to perennial lakes and rivers (Figures 2 and 3; Table 1). During the receding season, most turtles used River Channel (RC), Bay Beach (BB) and Open Flooded Forest Mixed (OFFM) habitats; however, during the dry season their most frequent use was nearby BB areas. During the flooding season, we noticed most of the monitored animals using OFFM and Flooded Forest (FF), the same habitats that were used during the full season (Table 1; Figure 3). Three monitored turtles were observed outside the boundaries of REBIO do Rio Trombetas near quilombola communities.

Distances Traveled

The average monthly distance traveled (AMD) was 5 ± 4.27 km, varying from 1 to 30 km a month. The mean total distance traveled (DT) by the eight subadult females was 34.51 ± 24.2 km (7.61–73.82 km). Notably, the period of reduced movement coincided with the

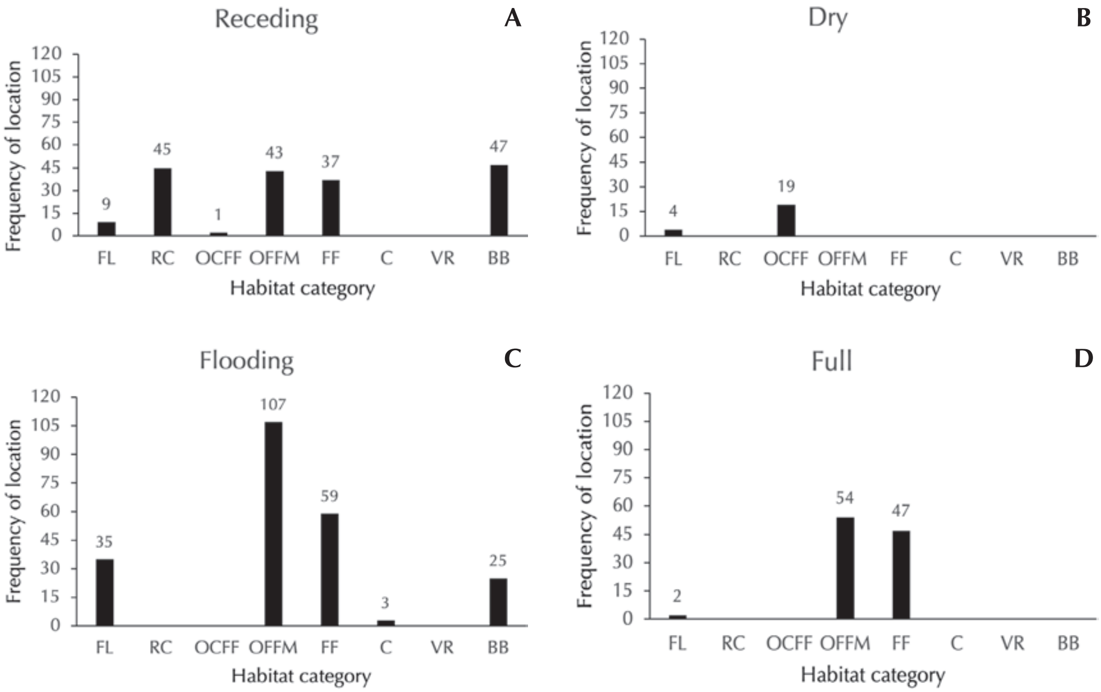

Figure 3. Frequency of habitat categories used by the young females of *P. expansa* per season in the Trombetas River Biological Reserve (REBIO do Rio Trombetas), Pará state, northern Brazil. BB: Bay beach; C: Cano; FF: Flooded forest; FL: Freshwater lake; OCFF: Open cuiarana flooded forest; OFFM: Open flooded forest mixed; RC: River channel; VR: Vegetated ravine.

high-water levels in the Trombetas river (Figures 2 and 4) with a negative correlation between river water level and DT ($F = 4.8715$, $df = 9$, $p < 0.05$).

Home Range

The mean values of the home range for female subadults varied depending on the estimator applied (Table 2; Figures 5 and 6). The linear home range (LHR) varied from 2.13–12.93 km (6.92 ± 3.70 km). However, using the estimators, we observed that the home range size of the young turtles monitored varied from 0.59 (MCP) to 293.43 km^2 (AKDE) with no significative difference among the home range estimations ($F = 0.922$, $df = 8$, $p = 0.5046$). The larger areas were estimated with AKDE, followed by KDE and MCP (Table 2). In August, we noticed young females near the aggregation of adult females by the nesting sites. On more than 20 occasions, subadult females were observed in proximity (≤ 30 m) to groups of adult females of *P. expansa* nesting on beaches (Figure 6). Notably, one of these large females equipped with radio-transmitters and monitored during a simultaneous study (Ferrara *et al*. 2013) was observed floating with a subadult female near the nesting site. The MCP for these four adult females with transmitters varied from 0.63 to 33.16 km^2 (10.97 ± 7.58 km^2). The KDE for these females ranged from 7.9 to 172.7 km^2 (98.1 ± 34.9 km^2). There was no significant difference between the MCP ($U = 14$, $Z = 0.25476$, $p = 0.7989$) nor the KDE ($U = 7$, $Z = 1.4437$, $p = 0.14884$) of adult females in comparison to the young females (Figures 5 and 6).

Figure 4. Movement (mean monthly distance traveled) by the young females of *Podocnemis expansa* (orange bars) and monthly river level (blue line) at the REBIO Trombetas, Pará state, northern Brazil.

Home Range Overlap

The overlap analysis using KDE$^{95\%}$ revealed distinct patterns of spatial use among female turtles. Adult females exhibited higher mutual overlap (0.52 ± 0.09), with several pairwise values above 0.25 (ranging from 0.26–0.89), indicating shared or adjacent core activity areas. In contrast, subadult females showed none to lower overlap among themselves (ranging from 0.00–0.37), with median value of 0.14 ± 0.02.

Table 2. The home range of the subadult radio-tracked females of the Giant South American River Turtle (*Podocnemis expansa*) monitored from October 2010 to September 2011 in the Trombetas River Biological Reserve, Pará state, northern Brazil. LHR: linear home range; MCP: Minimum Convex Polygon; KDE: Kernel Density Estimator; AKDE: Autocorrelated Kernel Density Estimator. The measurements are shown in km^2, except for LHR in km.

	LHR	MCP 95%	KDE 95%	AKDE 95%
Mean ± SD	6.92 ± 3.7	13.63 ± 13.86	36.84 ± 33.25	64.51 ± 95.91
Range	2.13–12.93	0.59–42.60	3.09–99.71	2.40–293.43

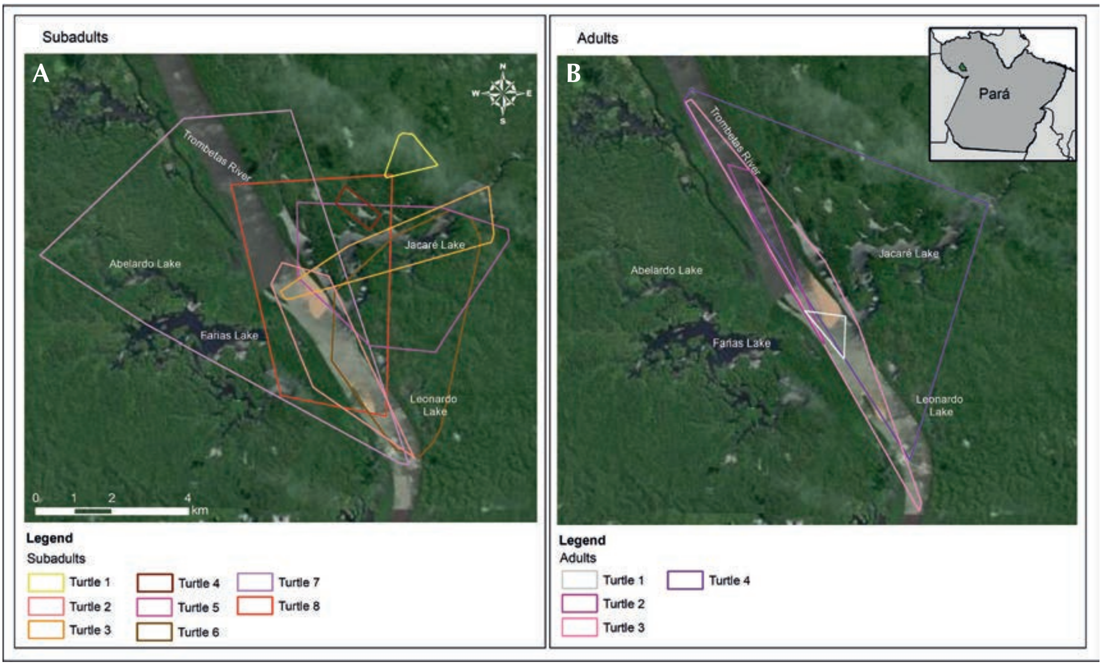

Figure 5. Minimum Convex Polygon (MCP$^{95\%}$) of subadult females of *Podocnemis expansa* (**A**) and exploratory MCP$^{95\%}$ for some adult females (**B**) radio-tracked at REBIO Trombetas, Pará state, northern Brazil.

Interactions between age groups were also observed with 75% of adult females overlapping with at least one subadult, while approximately 44% of subadults shared moderate to high spatial overlap with an adult (0.23 ± 0.03; 0.00–0.68). Interestingly, one of the smallest subadult individuals (CL = 22 cm and BM = 1.72 kg) exhibited relatively high home range overlap with two adult females, with values of 0.68 and 0.36, respectively (Figure 6).

Discussion

Female subadults of *Podocnemis expansa* exhibited distinct patterns of habitat use throughout the hydrological cycle of the Trombetas River. In our study, females make larger movements during the nesting season (August, September, and November to December), which is linked to low water levels. This pattern was observed for adult females in other areas of Amazonia (Carneiro and Pezzuti 2015). Subadult female *P. expansa* turtles monitored over the 11 months in our study moved between the Trombetas River and three perennial lakes (Jacaré, Abelardo, and Farias) located near nesting beaches and flooded forest areas. During the rising water phase, they migrated to the igapós, where the dense tree trunks and shrubs provided both shelter and abundant food resources (Almeida *et al.* 1986, Balensiefer and Vogt 2006, Vogt 2008). Turtles frequently use flooded forests, which serve as a vital habitat for feeding and refuge (Fachín-Téran *et al.* 1995). *Podocnemis expansa* primarily feeds on vegetation (Fachín-Téran *et al.* 1995, Malvasio *et al.* 2003, Cunha *et al.* 2020), with the igapó forest serving as a vital source of fruits and seeds in its diet due to the relatively low abundance of aquatic macrophytes

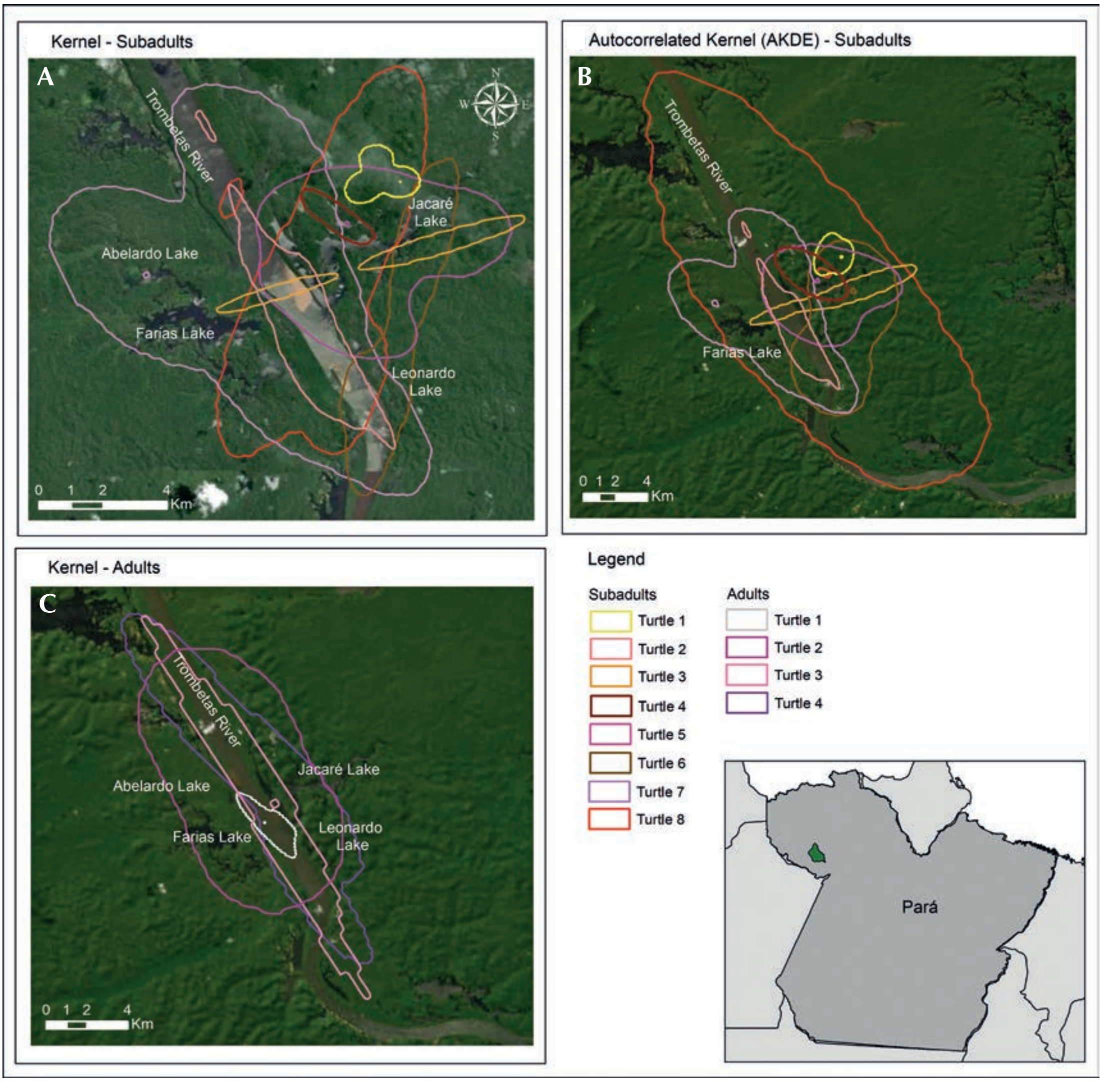

Figure 6. Kernels of the *Podocnemis expansa* turtles radio-tracked at the REBIO Trombetas, Pará state, northern Brazil. (**A**) Kernel Density Estimator (KDE95%) of subadult females; (**B**) Autocorrelated Kernel Density Estimator (AKDE) of subadult females; (**C**) Exploratory Kernel Density Estimator (KDE95%) of adult females.

in the Trombetas region (Vogt 2008, Cunha *et al.* 2020). In contrast, during the low water phase, they primarily inhabited lake and river environments, remaining in available water bodies such as lake pools and the river's main course with a preference for backwaters over the main river channel. This preference is likely influenced by the resistance provided by the sandbanks at nesting sites along the Trombetas River, making these areas more accessible and stable for small and young individuals. Consequently, the reduction in movement rate with increasing elevation and the subsequent flooding of the igapós, coupled with the abundant fruiting of vegetation (Armond 2008), suggests that the chelonians are finding food sources nearby and easily accessible. Similar behavior has been described in adult females of this species approximately two months after nesting in the same study area (Moreira and Vogt 1990, Castelblanco-Martinez *et al*. 2006), as well as in the Xingu River (Carneiro and Pezzuti 2015) and the Juruá River (Hinderaker 2021). For other species of Podocnemididae, such as *Podocnemis unifilis* Troschel, 1848, studies by Leão *et al*. (2019) and Hinderaker (2021) revealed similar habitat use, with individuals favoring the main river courses and lakes but using flooded forests less frequently than *P. expansa* during peak flooding.

The flood pulse remarkably influences the movement of aquatic vertebrates (Junk 1984, Junk *et al*. 1989). The effect of flood pulse on the movement of various species in the Amazonian rivers has been documented, including Peacock Bass (*Cichla* spp.; Hoeinghaus *et al*. 2003), Amazonian manatee [*Trichechus inunguis* (Natterer, 1883); Arraut *et al*. 2009)], the caimans, *Caiman crocodilus* (Linnaeus, 1758) (Silveira *et al.* 2010) and *Melanosuchus niger* (Spix, 1825) (Silveira *et al.* 2011), and turtles such as *Podocnemis sextuberculata* Cornalia, 1849 (Fachín-Terán *et al.* 2006) and *Podocnemis unifilis* (Leão *et al.* 2019). In the Trombetas region, the flood pulse results in an average annual elevation variation of up to six meters, which has a marked negative correlation with chelonian movement. Yet, subadults of *P. expansa* displayed movement patterns comparable to adult females of *Podocnemis erythrocephala* (Spix, 1824), *P. sextuberculata,* and *P. unifilis*, despite these species being smaller podocnemidid turtles within the Amazon biome (Fachín-Terán *et al.* 2006, Bernhard 2010, Cueva *et al.* 2018, Naveda-Rodríguez *et al.* 2018). Adult female *P. unifilis* exhibited an average displacement of 2.53 km (Leão *et al.* 2019) to approximately 16 km in the Napo River, Ecuador (Naveda-Rodrigues *et al.* 2018). *Podocnemis sextuberculata* displayed linear movements averaging 29.8 km in the Mamirauá Sustainable Development Reserve, Amazonas (AM; Fachín-Terán *et al.* 2006), and up to 60 km for one adult female in the Trombetas River, Pará (PA; Perrone *et al.* 2014).

Despite the growing availability of tracking technologies capable of addressing specific ecological questions (Guilhon *et al*. 2011), studies on *P. expansa* hatchlings and juveniles are scarce. Silva *et al*. (2017) recaptured a juvenile nine months after release, 38.47 km upstream in the Jaú River (AM), showing the displacement capacity of the species in early stages. Nevertheless, adults of *P. expansa* are known to display migration distances greater than 500 km, as documented in Xingu River (Carneiro and Pezzuti 2015, Carneiro 2017). On the Meta River, Colombia, Nieto-Vera (2024) reported a LHR average of 35.1 km (range 1.6–107.9 km) for adult females, while in the Trombetas River, adult females have been documented traveling over 45 km (Moreira and Vogt 1990) and 65 km downstream toward the Amazon River (Castelblanco-Martinez *et al.* 2006). In our study, adult females were frequently observed in nearby lakes several months after the nesting period. For instance, the radio signal of one adult female was recorded in Lake Jacaré during the flooding season of the Trombetas River. This finding suggests that long-distance migratory movements may not be a consistent behavior for all adult females of the species and that variations may exist among populations across the geographic range of the species and within a population.

As anticipated, the home range sizes estimated for subadult *P. expansa* in this study (average of 7 to 64.5 km²) were smaller than those reported for adult females. Although, we did not observe significant differences in home range sizes between adults and subadults, the adult home range estimates were based on a limited sample size. These estimates may not fully capture the critical areas used by adults and for that reason they are not presented here for comparison with the literature.

Studies on the home range of *P. expansa* reveal variability across different regions and estimator methods applied. Castelblanco-Martínez *et al*. (2006) reported a mean home range of 22.7 km² ($MCP^{95\%}$) for adult females in the Trombetas River. In the Juruá River, Hinderaker (2021) calculated the $FK^{95\%}$ area to be 748.81 km² for the species. Nieto-Vera (2024) reported an average of 8.22 km² with a $FK^{95\%}$ for adult females in the Meta River, Colombia. These differences underscore the impact of the size and age of an individual, ecological conditions, habitat availability, and methodological approaches on estimating home ranges for this species, highlighting the importance of developing conservation strategies tailored to specific contexts. Our results of home range for subadult *P. expansa* are more comparable to those observed for adults of *P. unifilis* than *P. expansa*. In the Juruá River, Hinderaker (2021) reported an average home range for *P. unifilis* of 311.67 km² using the $FK^{95\%}$ method, while the core area ($FK^{50\%}$) was estimated at 0.06 km². Naveda-Rodriguez *et al*. (2018) estimated home ranges for adult males and females in the Napo River, Ecuador, with $AKDE^{95\%}$ values averaging 5.2 km² (range 3.8–7.0 km²) and core areas ($AKDE^{50\%}$) averaging 1 km² (range 0.5–1.5 km²). In the Trombetas River the estimated $MCP^{100\%}$ of *P. unifilis* was 1.83 km², while the $KDE^{95\%}$ was 0.87 km² and core areas were 0.20 km² for females (Leão *et al*. 2019). These findings suggest that *P. unifilis* utilizes relatively smaller and more localized areas compared to *P. expansa*, with patterns resembling those of subadult females. In Brazil, *P. expansa* and *P. unifilis* are among the most extensively studied chelonian species, enabling comparisons. A recent study on *Podocnemis lewyana* Duméril, 1852 in Colombia reported a MCP of 7.5 km² and a $KDE^{95\%}$ of 3.71 km² for the species (Alzate-Estrada *et al.* 2020). Factors such as species-specific traits, population dynamics, and environmental conditions must be considered when drawing comparisons, as variations in home range size are influenced by habitat characteristics, resource distribution, and individual behavior. These results underscore the need to understand species-specific and regional differences to establish effective conservation and management strategies.

The presence of both subadult and adult females of *P. expansa* congregating near nesting beaches prior and during the nesting season is known across its geographical distribution (Ferrara 2012, Portelinha *et al*. 2014, Fonseca 2022). The simultaneous use of space by subadults and adults highlights the social behavior of the species, as previously observed among adult females and hatchlings during movement to the igapós after hatching (Ferrara *et al.* 2013). The arrival of the monitored subadults in the Trombetas River in August 2010–2011 coincided with the aggregation of adult female *P. expansa* pre-nesting (a couple of months before the beginning of nesting season in October). This finding indicates that social interactions are present and potentially meaningful across different age groups in *P. expansa.* Overall, spatial overlap appears to align with age or maturity, potentially reflecting differences in nesting behavior, habitat preferences, or social interactions. The overlap analysis revealed that adult females exhibited greater spatial overlap compared to subadult females, indicating shared or adjacent space use, likely associated with the utilization of sandbanks for nesting. The moderate to low overlap observed among subadult females may reflect more individualized or peripheral movement patterns. Our findings indicated that variations in movement patterns and habitat use were influenced by the size and maturity of individuals, resulting in different levels of home range overlap (Harestad and Bunnel 1979).

The proximity observed between different life stages of *P. expansa* may not solely result from the availability of resources, such as water and places for thermoregulation, but could indicate that subadult females are learning about reproductive behavior. The presence of nonmature *P. expansa* alongside adult females during the nesting season suggests that in addition to underwater communication during the aggregation period and vocalizations from young inside the nest (Ferrara *et al.* 2013), significant social interaction is taking place among females of different ages. The smallest female showed spatial affinity with adults, resembling patterns previously observed between adult females and recently emerged hatchlings (Ferrara *et al*. 2013). In a study tracking adult female *P. expansa* via satellite telemetry in the Xingu River, only one individual stayed near the nesting area (Carneiro and Pezzuti 2015). This aspect requires further investigation across the range of the species to determine whether it represents a local behavior and adaptation unique to the REBIO do Rio Trombetas.

The most suitable technique for assessing wildlife movement is an ongoing debate (Kenward 1987, Jacob and Rudran, 2006, Ouellette and Cardille 2011). For aquatic chelonians, the LHR method is commonly used to represent space utilization, particularly in Amazonia due to the linear and dendritic characteristics of water bodies (Morales-Verdeja and Vogt 1997, Fachín-Terán *et al.* 2006, Bernhard 2010, De la Ossa and Vogt 2011). In this study, there was no difference among the estimators. While the MCP method represented space use for most individuals, it failed to detect their use of river or lake environments, information revealed by the KDE and AKDE methods. Both KDE and AKDE methods better represented environmental use, particularly when individuals exhibited distinct and long-distance movement. As a movement-based method, AKDE effectively delineated core areas, useful for identifying critical shelter or feeding sites (Jacob and Rudran 2006). It was evident that employing multiple techniques to estimate space use is beneficial for understanding movement patterns. It is worth noting, however, that young individuals have not yet established their home ranges (Cagle 1944, Stickel 1950, Famelli 2013), which aligns with their learning behaviors observed in our study, especially linked to the reproductive and nesting season. This can also explain the absence of correlation between home range size and body size and the lack of difference among the estimators. We did not observe differences between adults and young females. This may happen due to the small sampling sizes of fixes of adult females and due to most of the monitoring happening during the nesting period when adult females are engaged in aggregations and nesting activities. Adult females of *P. expansa* have been documented traveling distances exceeding 45 km within a mere 48 hours (Moreira and Vogt 1990) or a maximum range of 352.9 m/day at the Xingu River (Carneiro and Pezzuti 2015), which surpasses the total distance covered by most of the monitored chelonians in this study.

According to Mogollones *et al*. (2010), protecting juveniles and adult turtles is the primary conservation action that could reverse a population decline. Understanding the needs of young female turtles is crucial for developing effective management and conservation strategies. Besides being heavily exploited for commercial purposes, *P. expansa* is at risk from the large-scale loss of natural habitats due to dam construction and climate change, which disrupts the flood pulse of rivers and lakes, with severe consequences for reproductive success and population sustainability (Castello and Macedo 2016, Eisemberg *et al.* 2016, Fagundes *et al.* 2018, Silva 2023). Caldas *et al*. (2023) indicated that key rivers for *P. expansa* have been fragmented because of dam construction, including those on the Xingu, Tocantins, and Araguaia Rivers. Another study employing distribution models for *P. expansa* revealed that it ranks as the fourth most impacted Amazonian turtle species by deforestation, primarily due to the potential loss of habitat along its distribution (Fagundes *et al.* 2018). Currently, *P. expansa* is in the process of being categorized as Endangered by the IUCN Red List of Threatened Species (C. R. Ferrara *et al.* pers. obs.). Knowledge about the social interactions between young adults and how they learn their behaviors is limited. Our study is the first to track young females of *Podocnemis expansa* and to suggest their potential social interactions with adults. We recommend the integration of long-term radio-tracking and satellite telemetry—either independently or in combination, as suggested by Guilhon *et al*. (2011)—alongside vocalization studies, to gain a deeper understanding of these interactions within their natural habitat. Satellite telemetry can also offer valuable insights into long-distance movements, spatial overlap between adults and subadults, and patterns of habitat use across different size classes and sexes. We recommend its implementation to improve our understanding of movement ecology and inform more effective conservation strategies. This information is vital for informing conservation and management strategies and to safeguard the studied species, particularly as environmental and climatic changes are expected to escalate (Oliveira *et al.* 2021, Marmontel *et al.* 2024).

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Variation of groove counts in *Caecilia thompsoni* (Gymnophiona: Caeciliidae) suggests gene flow between distant populations and calls for an updated diagnosis

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Abstract

Variation of groove counts in *Caecilia thompsoni* (Gymnophiona: Caeciliidae) suggests gene flow between distant populations and calls for an updated diagnosis. *Caecilia thompsoni* is a Colombian endemic species known from the Magdalena Valley that reaches a large body size (1767 mm maximum total length). To clarify some taxonomic concerns, we studied variation of the primary grooves, secondary grooves, grooves interrupted by the vent, total length, body width at mid-body, and length/width. We examined 77 specimens from four populations within the Magdalena Valley and found significant variation in the east–west direction for the humid (north) and dry (south) populations. We provide a redescription for the species and comment on the taxonomic challenges that currently affect the genus *Caecilia*.

Keywords: Annuli, Caecilians, Intraspecific variation, Morphology, Neotropical.

Resumen

Variación en los conteos de surcos de *Caecilia thompsoni* (Gymnophiona: Caeciliidae) sugiere flujo genético entre poblaciones distantes y amerita una diagnosis actualizada. *Caecilia thompsoni* es una especie endémica del Valle del Magdalena en Colombia y sobresale por alcanzar un gran tamaño corporal (1767 mm longitud total máxima). Con miras a resolver algunas dudas taxonómicas, estudiamos la variación de los surcos primarios, surcos secundarios, surcos interrumpidos por la cloaca, longitud del cuerpo, ancho a la mitad del cuerpo y largo/ancho. Examinamos 77 ejemplares provenientes de cuatro poblaciones dentro del Valle del Magdalena y encontramos que hay variación significativa en sentido occidente-oriente en las poblaciones húmedas (norte) y secas (sur). Por último hacemos una redescripción para la especie y discutimos sobre las dificultades taxonómicas que afectan actualmente al género *Caecilia*.

Palabras clave: Anillos, Cecilias, Morfología, Neotropical, Variación intraespecífica.

Resumo

Variação na contagem de sulcos em *Caecilia thompsoni* (Gymnophiona: Caeciliidae) sugere fluxo gênico entre populações distantes e exige uma diagnose atualizada. *Caecilia thompsoni* é uma espécie endêmica da Colômbia conhecida do Vale do Magdalena que atinge um grande tamanho corporal (comprimento total máximo de 1767 mm). Para esclarecer alguns problemas taxonômicos,

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estudamos a variação dos sulcos primários, sulcos secundários, sulcos interrompidos pela cloaca, comprimento total, largura na metade do corpo e relação comprimento/largura. Examinamos 77 espécimes de quatro populações no Vale do Magdalena e encontramos variação significativa na direção leste-oeste para as populações úmidas (norte) e secas (sul). Fornecemos uma redescrição para a espécie e comentamos sobre os desafios taxonômicos que atualmente afetam o gênero *Caecilia*.

Palavras-chave: Anéis, Cecílias, Morfologia, Neotropical, Variação intraespecífica.

Introduction

Caecilia thompsoni Boulenger, 1902 is the world's largest Gymnophiona, reaching a remarkable total length of 1767 mm. The species inhabits the Magdalena Valley lowlands as well as the neighboring foothills of the Cordillera Central and Cordillera Oriental of Colombia (79–1600 m a.s.l.). The type locality is Villeta, Cundinamarca. Over half a century ago, Edward H. Taylor provided the last meaningful taxonomic revision of *Caecilia thompsoni* (Taylor 1968), providing a detailed redescription of this taxon and offering valuable insights into its intraspecific variation. The closing remarks of his account said that he had examined two (of 10) specimens (MCZ 24552 and ILB 24 [now MLS 24]), which he doubted were conspecifics of *C. thompsoni.* Taylor (1968) considered the totality of the material to conform to two groups: the first had a lower count of primary grooves (187–207), and the second group had a higher count (220–243); he considered the latter group to attain a larger size than the former, but he was wary of the limitations imposed by the few specimens at his disposal. Moreover, Taylor (1968) claimed that the holotype of *C. thompsoni* (BMNH 1946.9.5.13) belonged to the first group. Dunn (1942) also examined 10 specimens of *C. thompsoni,* but he included two specimens that we consider dubious because their localities are unconfirmed (AMNH 49976 from 'Bogotá?') or out of the Magdalena Valley, (BMNH 1902.5.29, 179 from Río Caquetá). Acosta-Galvis (2012) reported an undescribed species (*Caecilia* sp.) from the Tatacoa Desert in Neiva, Huila. He claimed it was similar in color to *C. thompsoni* and that it differed from *C. thompsoni* by presenting 181–203 (vs. 187–240) primary grooves and 11–32 (vs. 26–42) secondaries. We have examined the material from Tatacoa Desert (ICN 58393–58400 and 58409) and consider these specimens to be conspecifics of *C. thompsoni* because their measurements and meristics fall within the extent of variation that we now associate with this species. To assess the claims of an undescribed species by Taylor (1968) and Acosta-Galvis (2012) and to understand the variation of characters presented by these authors, we studied groove counts of 77 specimens that cover the full distribution range of the species in the Magdalena Valley of Colombia.

Materials and Methods

We examined a total of 77 specimens of *Caecilia thompsoni* (Appendix I) and recorded their counts of primary grooves, secondary grooves, grooves interrupted by the vent, total length, width at mid-body, and length divided by width to assess the variation of these morphological characters (Tables 1 and 2). Meristics and measurements of the holotype of *C. thompsoni* were taken from Taylor (1968), and the number of grooves interrupted by the vent was determined via photographs. All groove counts were performed under a stereoscope and repeated at least twice for each individual. Entomological pins were used to demark and differentiate primary and secondary grooves to avoid misrepresenting their individual counts. Careful incisions to the mouth's commissure were performed using a razor blade in order to fully open the mouth and access dentition, choanae, and tongue when necessary. A sharp pin was used to open the pockets where the grooves concealed their dermal scales; once these were obtained, they were described in shape and size and put back in their respective positions so no material would be lost or damaged. Subdermal scales were sought by partial dissection of the annuli close to the mid-body point and removing a section of epidermis to expose the connective tissue and determine their presence or absence. Sex was determined through direct examination of gonads by performing a ventral longitudinal incision posterior to the mid-body point and prior to the vent to look for testes in males and ovaries in females. If mature testes or ovaries were found, these individuals were considered adults. Specimens with weakly calcified teeth, incomplete dentition, undefined nuchal collars, and total length usually under 400 mm were considered juveniles. All measurements were performed under a stereoscope; these were taken to the nearest 0.1 mm using a Neiko digital caliper, and a metric measuring tape was used to determine total length.

Table 1. Variation of primary grooves, secondary grooves, grooves interrupted by the vent, total length, body width at mid-body point, and attenuation index (i.e., length divided by width), of adult males, adult females, and juveniles of *Caecilia thompsoni*. Measurements and meristic data are provided as mean ± SE (range).

Characters	**Adult males (*N* = 33)**	**Adult females (*N* = 26)**	**Juveniles (*N* = 14)**
Primary grooves	221.1 ± 3.7 (174–267),	221.6 ± 5.1 (174–276)	190.2 ± 3.6 (169–223)
Secondary grooves	37.4 ± 1.4 (25–58)	37.5 ± 0.9 (28–46)	35.7 ± 2.1 (13–45)
Grooves interrupted by vent	4.3 ± 0.1 (2–6)	4.0 ± 0.3 (3–6)	3.1 ± 0.2 (2–5)
Total length (mm)	866.67 ± 54.6 (440–1666)	645.6 ± 47.73 (370–1310)	283.21 ± 24.72 (165–470)
Body width (mm)	13.33 ± 0.19 (4.8–15.1)	8.66 ± 0.5 (4.5–15.2)	4.5 ± 0.18 (3.4–8.2)
Length/width (times)	99.71 ± 2.57 (52.8–156.2)	88.75 ± 2.27 (44.6–119.1)	47.75 ± 0.59 (40.7–69.1)

Table 2. Variation of primary grooves, secondary grooves, grooves interrupted by the vent, total length, body width at mid-body point, and attenuation index (i.e., length divided by width), in the humid and dry populations of *Caecilia thompsoni*. Measurements and meristic data are provided as mean ± SE (range).

Characters/ Populations	**Dry east (*N* = 23)**	**Dry west (*N* = 16)**	**Humid east (*N* = 22)**	**Humid west (*N* = 16)**
Primary grooves	197.2 ± 3.7 (169–225)	204.6 ± 4.1 (184–231)	230.4 ± 5.3 (192–276)	226.4 ± 4.9 (200–259)
Secondary grooves	36.3 ± 1.5 (13–45)	35.3 ± 2.6 (16–56)	35.8 ± 0.9 (26–43)	37.5 ± 2.0 (28–58)
Grooves interrupted by vent	3.8 ± 0.2 (2–6)	4.0 ± 0.2 (3–6)	5.1 ± 0.1 (5–6)	4.2 ± 0.3 (2–6)
Total length (mm)	575.2 ± 66.9 (220–1180)	749.4 ± 59.1 (340–1040)	698.2 ± 75.8 (370–1550)	736.3 ± 98.6 (165–1666)
Body width (mm)	8.8 ± 0.6 (4.7–14.3)	9.7 ± 0.8 (4.8–15.5)	8.1 ± 0.7 (4.5–20.7)	7.9 ± 0.6 (3.4–15.1)
Length/width (times)	61.1 ± 3.6 (40.7–97)	79.2 ± 5.5 (41.4–122)	86.9 ± 4.5 (53.1–128.4)	88.1 ± 6.9 (45–156.2)

Museum abbreviations are as follows: Colección de Anfibios, Museo de Historia Natural, Universidad de Los Andes, Bogotá, D.C. (ANDES-A), Colección de Anfibios, Colecciones Zoológicas, Universidad del Tolima, Ibagué, Tolima (CZUT-A), Colección de Anfibios, Instituto Alexander von Humboldt, Villa de Leyva, Boyacá (IAvH-Am), Colección de Anfibios, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, D.C. (ICN), Museo de Herpetología, Colección de Anfibios, Museo La Salle, Bogotá, D.C. (MLS), Museo de Herpetología, Universidad de Antioquia, Medellín, Antioquia (MHUA), Colección de Herpetología, Museo de Historia Natural, Universidad del Cauca, Popayán (MHUC), Colección de Anfibios, Pontificia Universidad Javeriana, Bogotá, D.C. (MUJ), Colección de Herpetología, Colección de Anfibios, Universidad Industrial de Santander, Bucaramanga (UIS-A), Universidad del Valle, Cali, Valle del Cauca (UVC), Natural History Museum in London, U.K (BMNH) and Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts, U.S.A. (MCZ).

Sampling Geographic Area

The Magdalena Valley of Colombia is a heterogeneous region located between the eastern slopes of the Cordillera Central and the western slopes of the Cordillera Oriental of Colombia; its northernmost extension reaches the Caribbean coast, while the Huila Department marks its southern limit. The Magdalena Valley is often subdivided into three basins: The Upper Magdalena Valley (southern Cundinamarca, southern Tolima, and Huila) to the south, the Middle Magdalena Valley (Antioquia, Boyacá, Caldas, northern Cundinamarca, and Santander), and the Lower Magdalena Valley to the north (Atlántico, Bolívar, Cesar, and Magdalena). The Lower and Middle Magdalena Valley differ notably from the Upper Magdalena Valley because these areas are predominantly humid lowland forest, while the Upper Magdalena Valley comprises dry tropical forests and the Tatacoa Desert (Acosta-Galvis 2012) (Figure 1). For the purposes of this study, we use environmental heterogeneity to subdivide the Magdalena Valley into northern (humid) and southern (dry) sections, marked roughly by 4.5°N in Tolima and Cundinamarca, as well as east and west of the Magdalena River (Figure 1).

Results

Our results, based on qualitative and quantitative analyses of external morphology of the specimens fail to support the Taylor (1968) and Acosta-Galvis (2012) hypothesis of an undescribed species allied to *C. thompsoni* in Magdalena Valley. Herein we provide a redescription for the species based on variation of the morphological characters studied in our sample.

Diagnosis

Adults 400–1767 mm total length (Arredondo Salgar 2007), body width at mid-body point 4.5–20.7 mm, attenuation index (length divided by width) 45–156.2 times, sexual dimorphism not evident in any characters studied (meristics and measurements summarized in Table 1). Snout rounded in profile, projected anteriorly in some specimens, interorbital distance smaller than distance from tip of snout to eye level, margins of upper lips concave and downturned from the commissure of the mouth to the level of the tentacular opening, eye barely visible or hidden under translucent epidermis, tentacular openings oval in outline, elevated above skin, positioned below and slightly anterior to nostril, closer to margin of mouth than to nostril, not visible in dorsal view but clearly visible in ventral and lateral view, head narrower than body.

First nuchal collar smaller than second, with faint transverse nuchal grooves dorsally, missing medially but notable and deep ventrally, the second nuchal collar wider and more evident than the first, bearing a faint nuchal groove

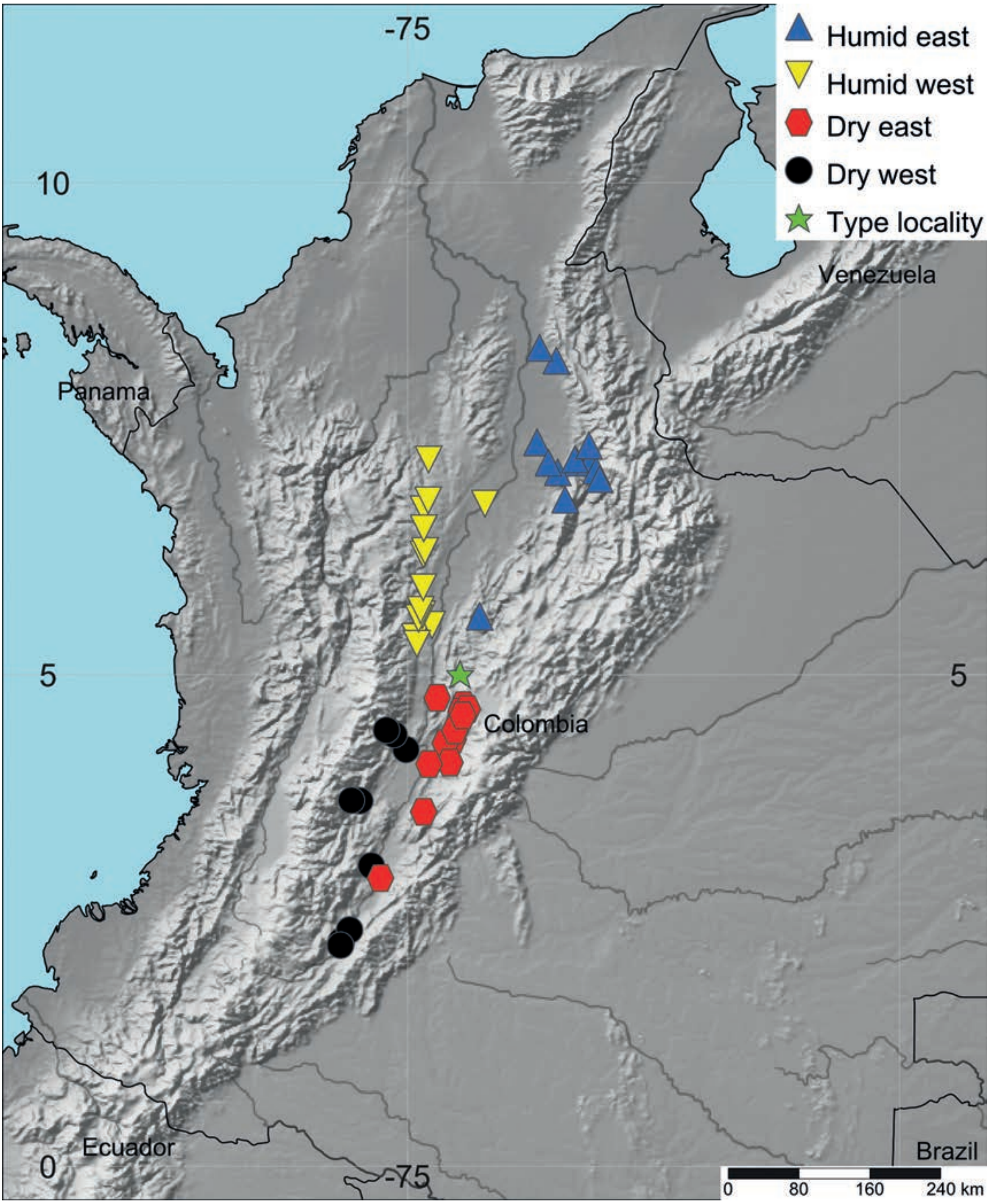

Figure 1. Map showing the geographic distribution of *Caecilia thompsoni* in the Magdalena Valley of Colombia. The humid east populations are represented by blue triangles, the humid west populations by yellow inverted triangles, the dry east populations by red rhombus, and the dry west populations by black circles. The type locality (Villeta, Cundinamarca) is indicated by a green star.

dorsally and slightly to flanks but absent ventrally; third nuchal groove incomplete ventrally. Body width increases considerably past nuchal collars, then gradually tapers toward mid-body point, where width remains mostly constant until last portion proceeding terminus which tapers abruptly, only to regain considerable width at end of body, i.e., terminus is wider than collars. Primary grooves 174–276, secondary grooves 25–58; last 4–9 primary grooves fully encircle posterior end of body, vent small, transverse, light gray in coloration, situated in a slight depression (concave), with 4–5 anterior denticulations and 5–6 posterior ones, anal glands white and small. No terminal shield, terminus segmented throughout. Dermal scales present throughout the body, those obtained posterior to collars are subcircular in shape, dermal scales present toward the terminus quite large, thick, and subrectangular, i.e., curved at anterior end of scale but almost straight at posterior end of scale (fitting Taylor's 1972, Fig. 76 description of dermal scales); those found toward flanks are overall rounded and less thick. Subdermal scales scattered along the connective tissue of skin.

All teeth monocuspid, recurved backwards and well-spaced from one another. Four series present, premaxillary–maxillary series bears maximum of 10-1-10 teeth, thick, though not very sharp, small, and decreasing in size posteriorly; vomeropalatine series has maximum of 12–12 teeth, thin, long, and sharp; dentary series has maximum of 10–12 teeth; inner mandibular teeth 3–3, small, pointed and partly concealed by gums. Teeth replacement signs evident in gums. Choanae deep, subcircular in shape and widening medially, distance separating them slightly greater than diameter of one choana; protruding narial plugs same color as tongue.

Coloration in life mostly dark gray dorsally, lighter gray toward flanks and slightly lighter on ventral surfaces (Figure 2). In preservative the main body coloration light gray, somewhat darker on the dorsal surfaces and slightly lighter on the ventral surfaces; in contrast, the mouth, tentacle, nostril and vent have a light cream coloration. Grooves appear to attain slightly blue tonality in preservation. No apparent ventrolateral stripe (Figure 3).

Morphological Remarks

Taylor (1968) provided a good diagnosis and redescription of the species, but we found a few errors on his behalf. He included material from a locality outside the Magdalena Valley, a misidentified specimen of *Caecilia nigricans* Boulenger, 1902 from Valle del Cauca in the Pacific region of Colombia (Thornton collection no. 154). He included two photographs supposedly of *C. thompsoni* but his drawings and a radiograph are in fact of *C. nigricans* (another elongated congener from the Pacific region of Colombia, Ecuador, and Panama). Taylor (1968) also claimed that anal glands were found only in females (i.e., sexually dimorphic), but we have found them in both sexes, independent of body size or geography. All material of *C. thompsoni* examined by Taylor during his visit to MLS, Bogotá during the mid-1960's are adult males (i.e., MLS 21–24). Two specimens examined (ICN 60332–60333) are peculiar because they lack inner mandibular teeth. The other specimens have 1–1 to 3–3 inner mandibular teeth. Regarding variation in total length, we are aware of an (expected) overlap in juveniles and adults at around 400 mm total length (Table 1) and emphasize that TL should not be the only character used to determine the age of specimens. The best way to determine sex and age is direct examination of gonads (Serrano-Pérez and Ramírez-Pinilla 2020).

Distribution

Caecilia thompsoni has been regarded as a lowland species that ascends to moderate elevations in the foothills of the Cordillera Central and Cordillera Oriental (Lynch 2000); however, this geographic view must be

Figure 2. Chromatic variation of *Caecilia thompsoni*. (**A**) ICN 60332, from corregimiento Jerusalen, Sonsón, Antioquia (humid west); (**B**) ICN 60333, from Aguachica, Cesar (humid east); (**C**) ICN 58389, from El Agrado, Huila (dry west); (**D**) uncatalogued individual from Purificación, Tolima (dry east). Photographs by Giovanni Chaves-Portilla (A–B), Guido Fabian Medina-Rangel (C), and Francisco José López-López (D).

reevaluated because the bulk of the collections of *C. thompsoni* come from moderate elevations (400–1600 m a.s.l.) on the eastern slopes of the Cordillera Central and the western slopes of the Cordillera Oriental. Very few records occur between 79–150 m a.s.l., i.e., from the true lowlands of the Middle Magdalena Valley.

Perhaps this species is more common or prefers the ecological conditions presented by the foothills of the Cordilleras Central and Oriental rather than those offered by the lowlands of the Magdalena Valley (Figure 1). However, one should not discard field work effort biases among the foothills of the Cordilleras (more explored) and the Magdalena Valley lowlands.

Discussion

On the Status of Caecilia thompsoni

Taxonomically, we were unable to find morphological evidence to support the Taylor (1968) and Acosta-Galvis (2012) hypothesis of two species concealed under the name *Caecilia thompsoni*. Our results indicate that there are slight differences between the four populations defined, such as the highest counts of primary grooves and largest body sizes in both the humid east and humid west populations of *C. thompsoni* (Tables 1 and 2), but we interpret this as individual variation and not as support for the

Figure 3. *Caecilia thompsoni* from El Agrado, Huila (dry west) (ICN 58389). (**A–B**) dorsal and ventral view of the body; (**C–E**) dorsal, lateral, and ventral view of the terminus; (**F–H**) dorsal, lateral, and ventral view of the head and collars. Upper scale bar equals 12 mm; lower scale bar equals 6 mm.

description of a new species, at least not until further evidence is presented. The fact that groove counts have such a wide range of variation suggests that more emphasis should be put on finding new diagnostic and informative characters for caeciliid taxonomy (Tables 1–2). Wilson and Brown (1953) had already contemplated a scenario where intraspecific geographic variation—either concordant or discordant—could be better analyzed, described, and named (either as subspecies or geographic variants) depending on the number of diagnostic characters employed by taxonomists in their studies. The fact that no meaningful diagnostic characters were found in the cephalic, terminal, genital, dental, and scale morphology to divide *C. thompsoni* indicates that the species should be regarded as valid.

Geographic Variation

At present, only three of the 77 records (3.8%) of *C. thompsoni* come from the Magdalena Valley lowlands (79–106 m a.s.l.) in municipios Aguachica (Cesar), and Barrancabermeja and Puerto Wilches (Santander), thus ruling out the possibility of thoroughly discussing variation of the lowland populations (Table 2). Therefore, we decided to limit our remarks to the populations of *C. thompsoni* on the east and west side of the Magdalena Valley and for both humid and dry populations (i.e., north and south). In this zone (Andean foothills), there is a significant difference in the east–west (latitudinal) orientation that is discordant in groove counts with the wet–dry (north–south) orientation (Table 2).

The total count of primary grooves, total body length, and attenuation indexes (length/width) differ between the humid and dry populations of *C. thompsoni,* given that the humid populations attain higher counts of primary grooves and are overall longer and more slender than their counterparts from dry populations. The dry populations are stouter, shorter, and attain lower counts of primary grooves (Table 2). Moreover, variation of secondary grooves, body width at mid-body point, and grooves interrupted by the vent does not vary geographically and is rather consistent between the humid and dry populations (Table 2). We recognize differences in the total counts of primary grooves, total length, and attenuation indexes as morphological tendencies (i.e., individual variation) of the populations studied (Wilson and Brown, 1953).

We also recognize slight variations in the main body coloration of *C. thompsoni* because some individuals from the dry populations, namely El Agrado, (Huila) (ICN 58389), Carmen de Apicalá (ICN 58509), and Purificación, (Tolima) (uncatalogued), appear to be darker (almost black), while most specimens from the humid populations are lighter gray or purple instead (Figure 2). Some individuals of *C. thompsoni* (Figures 2B and 2D) have a more pronounced bicolored pattern (similar to that of *Caecilia goweri* Fernández-Roldán and Lynch, 2021 and *C. pachynema* Günther, 1859), where the ventral surfaces of the body, and to some extent the flanks, have a light cream or white pigmentation subdivided by the primary and secondary grooves while the dorsal surfaces are notably darker-colored. This ‘’bicolored pattern’’ is less common that than the ‘’unicolored pattern’’ (i.e, almost the same coloration on the dorsal and ventral surfaces), seen in most specimens such as those in Figures 2A and 2C. Nonetheless, we emphasize that we do not attribute diagnostic value to these color variants.

On the Taxonomic Challenges of the Genus Caecilia

Caecilian taxonomy has historically relied on groove counts to diagnose and describe new species, primarily because these are easy to count and to use in dichotomous keys, but also (and perhaps more importantly) because posterior to the nuchal collars, most primary grooves have a ‘one to one’ relation to each vertebra. This arrangement means that the number of primary grooves is almost always equivalent to or very similar to the number of vertebrae (Nussbaum and Wilkinson 1989). Secondary grooves, on the other hand, are seemingly more variable, to the extent that some species have individuals with and without secondary grooves [i.e., *Caecilia abitaguae* Dunn, 1942, *C. guntheri* Dunn, 1942, *C. subdermalis* Taylor, 1968, *C. occidentalis* Taylor, 1968, *C. orientalis* Taylor, 1968, and *C. pachynema,* while others consistently lack them (*C. atelolepis* Fernández-Roldán, Lynch, and Medina-Rangel, 2023, *C. caribea* Dunn, 1942, *C. corpulenta* Taylor, 1968, *C. degenerata* Dunn, 1942, *C. pulchraserrana* Acosta-Galvis, Torres, and Pulido-Santacruz, 2019, *C. macrodonta* Fernández-Roldán, Lynch, and Medina-Rangel, 2023, and various undescribed taxa)]. However, we have demonstrated that their intraspecific variation—at least within *C. thompsoni*—is more limited than that of the primary grooves (Table 2).

Perhaps the strong point of the present study is the large sample of specimens of *Caecilia thompsoni* that allowed a more detailed examination of intraspecific variation, which, as expected, is considerably greater than what Dunn (1942), Taylor (1968), and Lynch (2000) had previously reported (but see Taylor's 1968: 311 account of *Chthonerpeton viviparum* Parker and Wettstein, 1929, a Brazilian typhlonectid with a wide range of primary grooves and vertebrae). This study has made us question if perhaps most caecilian species that are poorly circumscribed are prone to have a wider range of variation with regard to groove counts than what was previously reported in the literature. We consider that (caeciliid) groove-based taxonomy has been stable for the most part because of the paucity at which new caecilian specimens are obtained and the slow rate at which taxonomic revisions are published.

We determined that six characters (primary grooves, secondary grooves, the number of grooves interrupted by the vent, total length, body width, and attenuation index (length divided by width)), might serve to confront the suggestion of Taylor (1968) and the implication (without arguments or data) of Acosta-Galvis (2012) that there could be an undescribed species under the name *C. thompsoni* (the data for our specimens is summarized in Tables 1 and 2). These data illustrate that no significant difference is available to separate dry and humid subdivisions of the Magdalena Valley and, furthermore, that variation is individual and discordant for the six characters. In the future, a study that includes multiple samples from the entire distribution range of the species would provide insight into the population dynamics of *C. thompsoni*. For now, following Wilson and Brown (1953), we infer genetic flow throughout the distribution of *C. thompsoni* and recognize no taxonomic subdivisions.

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Appendix I. *Specimens examined. Countries appear in bold caps, departments written in italic type, localities given in plain text. F = adult females, M = adult males and J = juveniles, U = undetermined sex.*

Caecilia thompsoni (*N* = 77) (33 males and 26 females). **COLOMBIA:** *Antioquia:* San Carlos, Juanes, Puente Roto (1500 m a.s.l.): MHUA 7115 (F), Quebrada El Jordán (900 m a.s.l.): MHUA 7192 (F), San Roque, Estación Piscícola Universidad de Antioquia (1450 m a.s.l.): MHUA 5157 (F), Segovia, vereda Calavera (210 m a.s.l.): ICN 59586 (M), Sonsón, corregimiento Jerusalén, camino al Reserve Río Claro (385 m a.s.l.): ICN 60332 (M), Vegachi, vereda San Rafael (970 m a.s.l.): ICN 59664 (M)–65 (F), Yolombó, vereda vereda Santa Ana (977 m a.s.l.): ICN 58438 (J), Yondó, El Silencio (106 m a.s.l.): ICN 58437 (M). *Boyacá:* Humbo, Estación de Policía de Humbo (1035 m a.s.l.): MLS 21–22 (M). *Caldas:* Norcasia, Rio Manso (400 m a.s.l.): ANDES-A 4458 (J) and MUJ 7368 (M), La Miel (735 m a.s.l.): IavH 9688 (M), Samaná (500 m a.s.l.): ICN 41233 (J), La Victoria (510 m a.s.l.): ICN 43668 (M), MHUA 4432 (M) and 4247 (M). *Cesar:* Aguachica (79 m a.s.l.): ICN 60333 (M), Río de Oro, quebrada Peralonso (215 m a.s.l.): ICN 58392 (F). *Cundinamarca:* Beltrán, vereda El Tabór (375 m a.s.l.): ICN 58441 (M), Mesitas del Colegio (1260 m a.s.l.): ANDES–A 1904 (F), Nilo (330 m a.s.l.): ICN 47997 (M), La Esperanza (1240 m a.s.l.): ICN 21431 (M), Nilo: Pueblo Nuevo, hacienda La Isla (816 m a.s.l.): ICN 11763 (M), Tena, La Gran Vía, finca Tacarcuna (1100 m a.s.l.): MUJ 3402 (M) and 3713 (M), La Mesa (1270 m a.s.l.): MLS 24 (M), Viotá, finca El Danubio (1100 m a.s.l.): MUJ 8933 (M), Villeta (1000 m a.s.l.): BMNH 1946.9.5.13. (F). *Huila:* El Agrado (770 m a.s.l.): ICN 58389 (M), MHUC 1131–32 (U), Neiva, Desierto de La Tatacoa (400 m a.s.l.): ICN 58393–58400 and 58409 (J), El Quimbo (936 m a.s.l.): ICN 58439 (F), Neiva, ecoreserva La Tribuna (515 m a.s.l): IavH 16719 (M). *Santander:* Barrancabermeja (115 m a.s.l): IavH 17819 (U), Lebrija (717 m a.s.l.): UIS-A 5189 (M), vereda Portugal, Finca La Armenia (1090 m a.s.l.): UIS-A 20 (F), Puerto Wilches (105 m a.s.l.): IavH 17017 (U), Rionegro (700 m a.s.l.): UIS-A 4938–40 and 4943–46 (F), Floridablanca, barrio Bucarica (912 m a.s.l.): UIS-A 5190 (F), San Vicente de Chucurí, vereda La Colorada (1543 m a.s.l.): UIS-A 5378 (M) and 6865 (F), Betulia, vereda Aguamieluda (185 m a.s.l.): UIS-A 5753 (F), Rionegro, hotel campestre El Portal (793 m a.s.l.): UIS-A 6201 (F), Piedecuesta, vereda Monterredondo (1000 m a.s.l.): UIS-A 6427 (F). *Tolima:* Carmen de Apicalá, vereda Cuatro Esquinas (335 m a.s.l.): ANDES-A 4462 (M) and ICN 58509 (F), Chaparral, casco urbano, sector La Marina (1089 m a.s.l.): MHUA 6611 (M) and MLS 23 (M), Dolores, San Andrés: CZUT 1620 (M), Ibagué, casco urbano (1100 m a.s.l.): CZUT 277 (F)–78 (M), 1621 (F), 1622 (M), 1623 (M), 1625 (F), 2013 (J), MLS 34 (M), UVC 15666 (F), Icononzo, vereda Cafrerías (1320 m a.s.l.): ANDES-A 4448–49 (M), San Luis, hacienda Los Pijaos: CZUT 1624 (J) (465 m a.s.l.).

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In-stream movement of *Ambystoma altamirani* (Caudata: Ambystomatidae) from the Arroyo los Axolotes, Mexico

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Abstract

In-stream movement of *Ambystoma altamirani* (Caudata: Ambystomatidae) from the Arroyo los Axolotes, Mexico. Knowledge of the movements of amphibians can be useful in developing conservation strategies. We studied the movement of *Ambystoma altamirani,* a Mexican endemic salamander, in a montane stream near Mexico City, Mexico. We obtained movement estimates from 35 recaptured male, female, and juvenile salamanders. The majority of individuals moved during the study. Mean movements were > 250 m, with several individuals having movements > 500 m. Movements in *A. altamirani* did not have an upstream or downstream bias. Movement patterns (i.e., direction, mean distance, mean net displacement) did not differ among males, females, and juveniles. However, there was a tendency for the direction of net displacement to differ among males, females, and juveniles. Individuals that moved did not differ in snout–vent length (SVL) growth rate, initial SVL, or initial body mass (BM) from those that did not move; however, they showed greater BM change than those that did not move. We found that *A. altamirani* moved more than we had predicted. Our results suggest that maintaining aquatic and terrestrial conditions in the Arroyo los Axolotes to allow movements may be crucial in conserving this species.

Keywords: Amphibians, Downstream, Moviment patterns, Salamanders, Upstream.

Resumo

Movimentação de *Ambystoma altamirani* (Caudata: Ambystomatidae) dentro de um riacho de Arroyo los Axolotes, México. O conhecimento dos movimentos dos anfíbios pode ser útil no desenvolvimento de estratégias de conservação. Estudamos a movimentação de *Ambystoma altamirani*, uma salamandra endêmica mexicana, em um riacho montanhoso próximo à Cidade do México, México. Obtivemos estimativas da movimentação de 35 salamandras machos, fêmeas e juvenis recapturados. A maioria dos indivíduos movimentou-se durante o estudo. Os movimentos

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médios foram > 250 m, com vários indivíduos com movimentos > 500 m. Os movimentos de *A. altamirani* não mostraram uma tendência a montante ou a jusante. Os padrões de movimentação (ou seja, direção, distância média, deslocamento médio da rede) não diferiram entre machos, fêmeas e juvenis. No entanto, houve uma tendência de que a direção do deslocamento da rede fosse diferente entre machos, fêmeas e jovens. Os indivíduos que se deslocaram não diferiram em relação à taxa de crescimento do comprimento rostro-cloacal (SVL), SVL inicial ou massa corporal inicial (BM) daqueles que não se deslocaram; no entanto, apresentaram maior mudança na BM do que aqueles que não se deslocaram. Descobrimos que *A. altamirani* movimentou-se mais do que havíamos previsto. Nossos resultados sugerem que a manutenção das condições aquáticas e terrestres no Arroyo los Axolotes que permitem os movimentos pode ser crucial para a conservação dessa espécie.

Palavras-chave: Anfíbios, Jusante, Montante, Padrões de movimentação, Salamandras.

Introduction

Understanding how and why amphibians move and the consequences of these movements can aid in developing conservation strategies (Bailey and Muths 2019). Movement of salamanders in streams is often examined in the context of upstream and downstream movement, with species showing upstream-biased movements (Lowe 2010, Cosentino *et al.* 2009, Campbell Grant *et al.* 2010), downstream-biased movements (Bruce 1986, Schafft *et al.* 2022), or equal movement upstream and downstream (Bendik *et al.* 2016). In addition to the direction of movement, the distance of in-stream movements by salamanders can vary dramatically among species. Many species of stream-dwelling salamanders show relatively short movements, with movements typically < 10 m (e.g., Barthalmus and Bellis 1972, Holomuzki 1991, Cosentino *et al.* 2009). However, some species show longer movements. For example, *Eurycea tonkawae* Chippindale, Price, Wiens, and Hillis, 2000 often made movements of > 15 m in a stream, frequently recolonizing sites that had dried (Bendik *et al.* 2016), and *Gyrinophilus porphyriticus* (Green, 1827) can move up to 565 m along a -km stream (Lowe 2010).

Previous studies of Mexican stream-dwelling *Ambystoma* salamanders suggested that they do not move much along the streams they inhabit, due to low temperatures and poor terrestrial habitat matrix quality (little canopy cover), likely leading to a small degree of subpopulation genetic structure associated with substrates in the streams (Sunny *et al.* 2014). We examined the movement of a Mexican endemic salamander, *Ambystoma altamirani* Dugès, 1895, in the Arroyo los Axolotes, state of Mexico. *Ambystoma altamirani* occurs in streams in the mountains near Mexico City (Lemos-Espinal *et al.* 1999, Woolrich-Piña *et al.* 2017). The ecology and natural history of *A. altamirani* have been extensively studied in the Arroyo los Axolotes (Lemos-Espinal *et al.* 2016, Villarreal Hernández *et al.* 2019, 2020a,b, Gómez-Franco *et al.* 2022, Sánchez-Sánchez *et al.* 2022, Hernández-Luría *et al.* 2023, 2025). This species spends most, if not all, of its life in streams or immediately adjacent to streams (Lemos-Espinal *et al.* 1999). The abundance of *A. altamirani* along a stream appears to be driven more by in-stream factors (e.g., hydroperiod, total dissolved solids, stream width, bank height, water velocity, water temperature, and water depth) than terrestrial factors (e.g., presence of livestock, distance to forest) (Gómez-Franco *et al.* 2022). We specifically studied the distance and the direction (upstream or downstream) moved by individuals. We also determined the relation between distance and direction moved and sex, stage, size, and growth of the individuals.

Materials and Methods

Study Area

Arroyo los Axolotes is a stream located along the southern edge of an extensive 100-ha pasture called the Llano Las Navajas, which is surrounded by a *Pinus hartwegii* Lindl. forest in the municipality of Isidro Fabela, Sierra de las Cruces, State of Mexico (19°32'12.2" N, 99°29'52.7" W, datum WGS84, 3479 m a.s.l.). Between May and November, surface water flows in two main channels; however, between December and April it is limited to a single channel with several shallow pools (about ≤ 3.5 m diameter, ≤ 1 m depth) connected by branches of the main channel that have very slow water flow. Livestock grazing and human recreational activities are concentrated on the northern edge of the pasture (Gómez-Franco *et al.* 2022).

Field Methods

From 25 February 2022 to 13 April 2023, we visited the study area once or twice per month, usually 15 d apart. We searched a 1-km section of the Arroyo los Axolotes for *A. altamirani,* capturing salamanders using dipnets. We recorded each captured individual's location using a mobile global positioning system [GPS] unit (Garmin Etrex Venture GPS, Olathe, Kansas, USA; accuracy to within < 15 m). We measured SVL of each individual to the nearest 1 mm using a transparent plastic ruler and weighed each salamander to the nearest 0.001 g using a portable digital pocket scale (Zencro® 0.001–50 g; Dongguan Zencro Industrial Co., Dongguan, China). Adult males were identified by the presence of a bulge on each side of the tail near the cloaca (i.e., swollen cloacal lips) and adult females by the absence of this bulge (Brandon and Altig 1973). Males with swollen cloacal lips were observed throughout the year, suggesting that this is a reasonable way to sex individuals in this population. Adults of *A. altamirani* at the Arroyo los Axolotes generally lack gills once they complete metamorphosis (Villanueva Camacho *et al.* 2020). Size class was assessed based on SVL (20–34 mm: larvae; 35–64 mm: juveniles, and > 64 mm: adults) (Lemos-Espinal *et al.* 2016, Villanueva-Camacho *et al.* 2020, Gómez-Franco *et al.* 2022). During each survey, we used Visible Implant Elastomer (Northwest Marine Technology, Inc., Anacortes, Washington, USA) to mark any unmarked individuals using individual codes created from VIE color and location on the body, using up to four marks (MacNeil *et al.* 2011). The VIE injector was sterilized between uses to prevent potential contamination among individuals. We did not detect any loss of VIE from the salamanders (i.e., no incomplete marks that would have suggested the loss of VIEs). Previous studies using VIE to mark aquatic salamanders have generally found high retention and readability rates for VIEs (e.g., Phillips and Fries 2009, Moon *et al.* 2022, Knapp *et al.* 2023), and VIEs have been successfully used in *A. altamirani* (Guerrero de la Paz *et al.* 2020). We returned salamanders to their capture location and monitored their activity for a few minutes to ensure they were in good condition.

Data Analysis

We used the distance between the coordinates taken at the time of capture and recapture of an individual to measure displacement, as well as direction (i.e., upstream or downstream) of any movement. Distance moved was calculated by summing the absolute value of all movements made by an individual for all recapture intervals. We calculated net displacement by assigning negative values to upstream movements and positive values to downstream movements. For example, if an individual moved 40 m upstream in one interval and 50 m downstream in the next interval the net displacement was -10 m and total movement was 90 m. We also calculated the median net displacement, skewness, and kurtosis of the distribution of net displacements. We calculated SVL growth rate and BM change by subtracting the final SVL or BM by the initial SVL or BM and dividing by the number of days between captures.

We compared frequencies of movements (upstream, downstream, and not moved) between males and females using a chi-square test or Fisher's exact test. We tested whether mean distance moved and mean net displacement differed among males, females, and juveniles using analyses of variance. We used linear regressions to examine the relationships between distance moved and net displacement with initial SVL. We used a two-way ANOVA to examine if the initial SVL, mean SVL growth rate, and mean BM change differed as a function of movement status (moved or not moved) and sex. Means are given ± 1 SE.

Results

During our surveys we marked a total of 194 individual *A. altamirani* throughout the study section, which provides a minimum density estimate of 0.194 individuals m^{-1}. We recaptured 35 individuals (4 juveniles, 22 females, 9 males) an average of 2.3 ± 0.1 times (median = 2), and the mean number of recaptures did not differ among males, females, and juveniles ($F_{2,32} = 0.19, p = 0.83$).

The distribution of net displacements for all individuals is given in Figure 1. Mean net displacement for all individuals was 41.66 ± 44.89 m (positive = downstream); however, the median was 0. The skewness was -0.12 and kurtosis was 1.16.

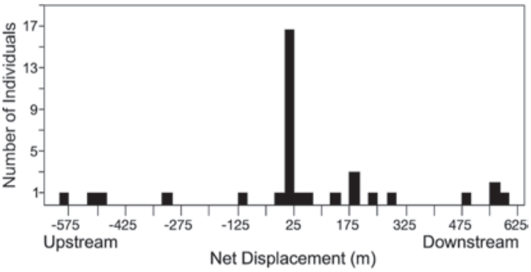

Figure 1. The distribution of net displacements of *Ambystoma altamirani* along the Arroyo los Axolotes.

Males and females showed similar frequencies of upstream, downstream, and no movements (Table 1; $c^2_2 = 2.34, p = 0.31$) and upstream vs downstream movement (Fisher's exact test, $p = 0.33$). Overall, individuals showed no tendency to move upstream, downstream, or to not move (Table 1; $c^2_2 = 3.5$, $p = 0.17$). Of those individuals that did move, there was no preference to move upstream or downstream for all individuals (Table 1; $c^2_2 = 1.8$, $p = 0.18$), just females (Table 1; $c^2_1 = 0.33, p = 0.56$), or just males (Table 1; Fisher's exact test, $p = 0.28$).

Mean distance moved did not differ between males, females, and juveniles (Table 1; $F_{2,32} = 0.19, p = 0.83$). Mean net displacement also did not differ between males, females, and juveniles; however, it appears net displacement by juveniles was negative, males positive, and females near 0 (Table 1; $F_{2,32} = 2.09, p = 0.14$).

The distance moved was not related to the initial SVL of the salamander ($N = 35$, $r^2 = 0.024$, $p = 0.38$) nor was the distance moved per day ($N = 35, r^2 = 0.031, p = 0.31$). There was a tendency for net displacement to decrease with the initial SVL of the salamander (Figure 2; $N = 35, r^2 = 0.10, p = 0.065$).

The mean initial SVL of salamanders that moved and did not move did not differ (Figure 3A; $F_{1,27} = 0.30$, $p = 0.59$). Mean initial SVL did not differ between males and females (Figure 3A; $F_{1,27} = 0.09$, $p = 0.76$). The interaction between sex and movement was not significant (Figure 3A; $F_{1,27} = 0.91, p = 0.35$).

The mean initial BM of salamanders that moved and did not move did not differ (Figure 3B; $F_{1,27} = 0.32$, $p = 0.57$). Mean initial BM did not differ between males and females (Figure 3B; $F_{1,27} = 0.03$, $p = 0.87$). The interaction between sex and movement was not significant (Figure 3B; $F_{1,27} = 2.68, p = 0.11$).

Mean SVL growth rate did not differ between males and females (Figure 3C; $F_{1,27} = 0.66$, $p = 0.42$). The mean SVL growth rate of

Table 1. The number of male, female, juvenile, and all *Ambystoma altamirani* individuals that moved upstream, downstream, or did not move over the course of the study in the Arroyo los Axolotes, Mexico. Mean ± SE distance moved (with and without individuals showing no movement) and mean ± SE net displacement (+ = downstream, - = upstream) are also given. *One male moved upstream and downstream to return to its original location.

	Males	**Females**	**Juveniles**	**All**
Upstream	1*	5	1	7*
Downstream	6*	7	0	13*
Not moved	3	10	3	16
Total	9	22	4	35
Mean distance moved (m)	200.4 ± 72.4 (9)	161.2 ± 45.6(22)	125 ± 125 (4)	167.1 ± 36.0 (35)
Mean distance moved (no 0 values) (m)	300.7 ± 81.0 (6)	295.5 ± 60.0 (12)	500	307.9 ± 45.9 m (19)
Mean net displacement (m)	172.4 ± 75.3 (9)	18.4 ± 57.5 (22)	-125 ± 125 (4)	41.7 ± 44.9 (35)

salamanders that moved and did not move did not differ (Figure 3C; $F_{1,27} = 0.65$, $p = 0.43$). The interaction between sex and movement was not significant (Figure 3C; $F_{1,27} = 0.08$, $p = 0.78$).

Mean BM change did not differ between males and females (Figure 3D; $F_{1,27} = 1.47$, $p = 0.24$). Mean BM change of salamanders that moved was greater than that of salamanders that did not move (Figure 3D; $F_{1,27} = 5.14$, $p = 0.032$). The interaction between sex and movement was not significant (Figure 3D; $F_{1,27} = 0.02$, $p = 0.89$).

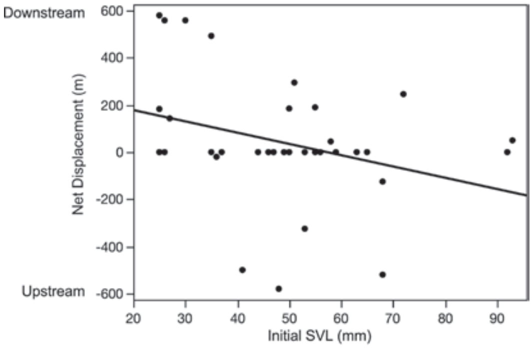

Figure 2. The relationship between initial SVL and net displacement of *Ambystoma altamirani* in the Arroyo los Axolotes.

Discussion

One of our most surprising results is how much *A. altamirani* moved over the course of the study. More than one-half of all individuals moved during the study. In addition, the distances moved were substantial, with mean movements being > 250 m, and several > 500 m. This result contrasts with previous studies on movement of *Ambystoma* in streams. Holomuzki (1991) found evidence for relatively limited dispersal within a stream in *Ambystoma barbouri* Kraus and Petranka, 1989, with 78% of larvae recaptured within 1.5 m of the release point. *Ambystoma leorae* Dugès, 1895 showed a small degree of subpopulation genetic structure associated with substrates in the streams, possibly due to limited mobility of individuals along the stream (Sunny *et al.* 2014).

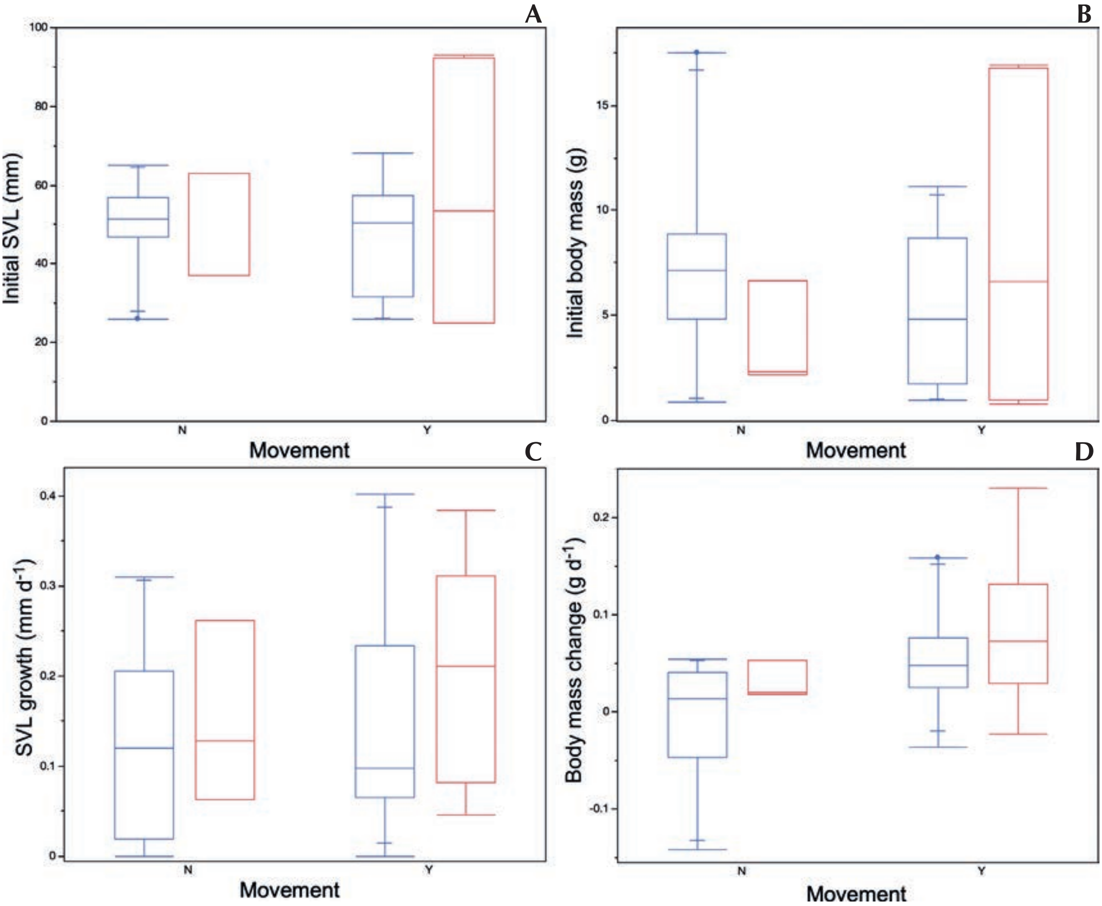

Figure 3. Box and whisker quantile plot of (**A**) initial SVL, (**B**) initial body mass, (**C**) SVL growth rate, and (**D**) BM change of male (blue) and female (red) *Ambystoma altamirani* along the Arroyo los Axolotes that did and did not move over the course of the study.

Ambystoma altamirani in the Arroyo los Axolotes moved both upstream and downstream with no apparent bias. We cannot determine whether these movements took place entirely in the water of if terrestrial movement was involved. For example, movement of the stream-dwelling salamanders, *Desmognathus fuscus* (Green, 1818) and *D. monticola* Dunn, 1916, occurred both in-stream and overland (Campbell Grant *et al.* 2010). For juveniles and gilled individuals, terrestrial movements would seem unlikely. We do not know this for certain, but we have never observed juveniles or gilled individuals on land in this well-studied population. The serpentine nature of the Arroyo los Axolotes is conducive to short overland movements by adults without gills. The distances we report are straight-line distances and would be much longer if movements were strictly aquatic. Using radiotracking could be informative to determine the paths used and whether terrestrial movements are undertaken. Such information could be used to identify whether particular terrestrial habitats might facilitate or hinder overland movements or if all movements are in the stream channel (see Wang *et al.* 2009). Determining this is important to maintain connectivity within populations by ensuring appropriate terrestrial and aquatic habitats.

We did not find statistically significant differences in movement patterns (i.e., direction, mean distance, mean net displacement) between males, females, and juveniles. However, there were tendencies for differences in the direction of the net displacement among males, females, and juveniles. Greater sample sizes, especially of males and juveniles, may permit determination of whether those trends are or are not more strongly supported.

Ambystoma altamirani that moved showed greater BM change than those that did not move; however, they did not differ in SVL growth rate, initial SVL, or initial BM. These results suggest *A. altamirani* that moved may be moving to improve conditions (i.e., find more food, more favorable temperatures) that allow increase in body mass. For example, individuals of *Gyrinophilus porphyriticus* that dispersed farther along a stream had higher survival and growth rates, probably because the ability to settle in a good habitat increased with dispersal distance (Lowe 2010). Alternatively, the individuals who moved were those in better physical condition, and thus increased BM more, were more likely to move. Again, more fine-scale monitoring of movements using radiotelemetry or more frequent observations could help differentiate these options, as well as determine more accurately the total movement of each individual.

In conclusion, our results show that *A. altamirani* does not appear to be sedentary and indeed moves more than we had expected based on prior work on Mexican *Ambystoma* (e.g., Sunny *et al.* 2014). Such movement may permit greater admixture of genes. *Ambystoma leorae* in streams near Mexico City showed some small degree of subpopulation structure associated with substrates in the streams, possibly because individuals do not appear to move very much from specific sites along the stream (Sunny *et al.* 2014). Future studies should investigate habitat use and spatial ecology of the species and the implications of its high mobility (e.g., movement may allow greater admixture of genes, increase genetic diversity and structure), in order to evaluate if and how maintaining particular aquatic and terrestrial conditions in the Arroyo los Axolotes may favor the conservation of *A. altamirani.*

Acknowledgments

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Complex courtship of *Bokermannohyla capra* (Anura: Hylidae), a treefrog that inhabits lotic environments

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Abstract

Complex courtship of *Bokermannohyla capra* (Anura: Hylidae) a treefrog that inhabits lotic environments. *Bokermannohyla capra* is an endemic species of the Atlantic Forest whose biology and ecology are poorly understood. We studied the reproductive behavior of this species in an ecotonal Atlantic Forest–Caatinga area in south-central Bahia state. We collected data on habitat use, courtship, and calling behavior. Males of *Bokermannohyla capra* were observed calling at night, peaking between 19:00 and 21:00 h. We recorded reproductive activity in *B. capra* non-continuously throughout the period of the study, from February 2020 to September 2021, both in dry and rainy seasons. The number of males calling ranged from one to nine individuals. The vocalization microhabitat is vegetation along stream banks, but the species also uses bromeliads as vocalization sites and shelter. *Bokermannohyla capra* has an elaborate courtship with acoustic, tactile, and visual signals. The male guides the female to the spawning site, where they perform axillary amplexus; oviposition occurs in small puddles along stream banks. Like many species of the Cophomantini tribe, *B. capra* also displays a repertoire of elaborate reproductive behaviors related to the occupation of lotic and noisy permanent environments. The data presented here expand our knowledge about the ecology and reproductive biology of *B. capra*. Our results also contribute to the knowledge of the taxonomic group to which the species belongs.

Keywords: Amphibians, Behavior, Communication, Cophomantini, Ecology, Reproduction.

Resumo

Corte complexa de *Bokermannohyla capra* (Anura: Hylidae), uma perereca de ambientes lóticos. *Bokermannohyla capra* é uma espécie endêmica da Mata Atlântica cuja biologia e ecologia ainda são pouco conhecidas. Para entendermos as características do seu comportamento reprodutivo, estudamos uma população da espécie em uma área ecotonal entre Mata Atlântica e Caatinga no

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centro-sul do estado da Bahia. Nós coletamos dados sobre os aspectos comportamentais de *B. capra*, incluindo informações sobre o uso do habitat, corte e vocalizações. Os machos de *B. capra* foram observados em atividade de vocalização noturna, com pico entre 19:00 e 21:00 h. A atividade reprodutiva foi registrada de forma não contínua durante todo o período de estudo (fevereiro de 2020 a setembro de 2021), tanto em meses considerados secos como em meses considerados chuvosos. O número de machos vocalizando variou de um a nove indivíduos. O micro-habitat de vocalização é a vegetação ao longo das margens dos riachos, mas a espécie também usa bromélias como local de vocalização e abrigo. *Bokermannohyla capra* apresenta uma corte elaborada com sinais acústicos, táteis e visuais. O macho guia a fêmea até o local de desova, onde realizam um amplexo axilar; a oviposição ocorre em pequenas poças ao longo das margens dos riachos. Como muitas espécies da tribo Cophomantini, *B. capra* também apresenta um repertório de elaborados comportamentos reprodutivos relacionados à ocupação de ambientes permanentes lóticos e ruidosos. Os dados aqui apresentados contribuem para a expansão do conhecimento sobre a ecologia e biologia reprodutiva de *B. capra*. Nossos resultados também enriquecem o conhecimento do grupo taxonômico ao qual a espécie pertence.

Palavras-chave: Anfíbios, Comportamento, Comunicação, Cophomantini, Ecologia, Reprodução.

Introduction

Natural history of a species includes data on its environmental use, diet, seasonality, population density, and reproductive cycle (Donnelly and Guyer 1994, Gally and Zina 2013, Almeida *et al*. 2019, Faraulo and Zina 2019, Oliveira *et al.* 2021). Reproduction is a crucial aspect that modulates a number of these descriptors, from the use of the environment to how species are distributed spatiotemporally within their range (Wells 1977, Donnelly and Guyer 1994, Pombal *et al*. 1994), which is particularly perceptible in taxonomic groups showing high reproductive diversity such as frogs (Haddad and Prado 2005).

In anurans reproduction is strongly influenced by both intrinsic factors (e.g., physiology and anatomy) and extrinsic environmental conditions (e.g., climate) (Pombal and Haddad 2005). Abiotic variables such as temperature, humidity, light intensity, and the availability and permanence of water bodies commonly regulate the duration and intensity of reproductive cycles (Rome *et al*. 1992, Stebbins and Cohen 1997, Wells 2007), as well as the selection of calling and oviposition sites (Duellman 1985, Resetarits Jr. 2005, Silva and Giaretta 2008). Given the close relationship between reproductive traits and climatic variability, most frog species exhibit a seasonal reproductive pattern (Wells 1977), with breeding activity typically triggered by the onset of optimal abiotic conditions that enhance the survival and development of eggs and tadpoles (Wells 2007).

Reproductive strategy can be defined as a combination of physiological, morphological, and behavioral attributes that act together to optimize the offspring number under certain environmental conditions (Duellman and Trueb 1994). Such strategies involve the stimulation of the partner (Lantyer-Silva *et al*. 2014, Cayuela *et al*. 2017, Dias *et al.* 2021), which occurs through signals emitted by individuals capable of promoting a change in the behavior of the receptors when courting (Hebets and Papaj 2005, Zina and Haddad 2007, Lima *et al.* 2014, Centeno *et al*. 2015, de Sá *et al.* 2016).

Acoustic signaling is the primary mode of communication mediating intraspecific interactions in most anuran species (Ryan 2001, Alcock 2009), with the advertisement call being the most frequently emitted vocalization (Narins *et al*. 2006). Due to its species-specific nature, the advertisement call serves a dual function: it acts as a key mechanism for prezygotic reproductive isolation and constitutes a valuable taxonomic tool (Martins and Jim 2003, Köhler *et al*. 2017, Forti *et al*. 2019). While certain call traits display phylogenetic conservatism, others are modulated by the environmental context in which populations occur (Gingras *et al*. 2013, Zina and Haddad 2007). Variations in temporal and spectral parameters—such as the number of pulses per note and per call, note and call duration, inter-note intervals, and dominant frequency—may reflect population-specific characteristics (Napoli and Pimenta 2009), thereby contributing to a deeper understanding of the biogeographic and evolutionary history of taxa.

Courtship behavior is an interaction between a female and a male, usually resulting in females evaluating their partner before amplexus and oviposition (Zina *et al*. 2007, Lantyer-Silva *et al.* 2014). Courtship behavior patterns and complexity vary in social organization, reproductive period, and phylogeny (Zina and Haddad 2007, Centeno *et al.* 2015, Nali *et al*. 2022). In anurans, such behaviors generally include chemical, tactile, acoustic, or visual stimuli (see Zina and Haddad 2007, de Sá *et al.* 2016). The combined use of two or more types of these stimuli is called multimodal communication (Preininger *et al.* 2013, de Sá *et al.* 2016) and reinforces a message by exploring more than one sensory channel (Preininger *et al.* 2013, de Sá *et al.* 2016). The use of multimodal communication can facilitate mating by promoting a more accurate location of the partner or the area prepared by a male (see Haddad and Sawaya 2000, Zina and Haddad 2007).

Although we have several records of courtship behavior for some species of Cophomantini (Zina and Haddad 2007, Nali *et al*. 2022), this type of study is rare due to the sporadic nature of observing such behaviors. Observation of courtship behavior requires extensive field observations or favorable conditions that allow similar behaviors to be observed in a laboratory setting as reported for *Bokermannohyla ibitiguara* (Cardoso, 1983) by Nali *et al*. (2022), one of the few documented studies for this genus.

The genus *Bokermannohyla* currently comprises 31 species (Frost 2024), most of which inhabit lotic environments, generally at low population densities (Napoli and Pimenta 2009). Several species within the genus exhibit elaborate courtship behaviors (Nali and Prado 2012, Centeno *et al*. 2015); however, such behaviors remain undescribed for many others. *Bokermannohyla capra* Napoli and Pimenta, 2009 is geographically restricted to small forest fragments at high elevations in the Atlantic Forest of south-central Bahia (Frost 2024), where it occurs at low densities, typically utilizing vegetation along margins of small streams (Napoli and Pimenta 2009). Due to its narrow geographic range and low population density, *B. capra* is highly susceptible to the impacts of anthropogenic activities, particularly those related to land-use change, habitat fragmentation, and habitat loss (Napoli and Pimenta 2009). The lack of ecological and behavioral data, including aspects of its natural history, hampers a reliable assessment of the conservation status of this species.

Herein, we describe the reproductive behavior of *B. capra,* report information on its use of the environment, seasonality, and attributes of the advertisement call. Our data increases our understanding of the biology of this species and others in the genus, thus allowing the formulation of hypotheses about the evolution of some aspects of their natural history.

Materials and Methods

Study Site

The study was carried out in Morro do Mara (MM) (13°53'42.72" S, 39°57'35.64" W), an Atlantic Forest–Caatinga ecotonal and mountainous area in central-southern Bahia, located at the municipality of Jequié, northeastern Brazil (Figure 1). MM is a forest fragment situated at one of the highest elevations in the region, ranging from 560 to 960 m and covering an area of approximately 6.5 km². Unlike the

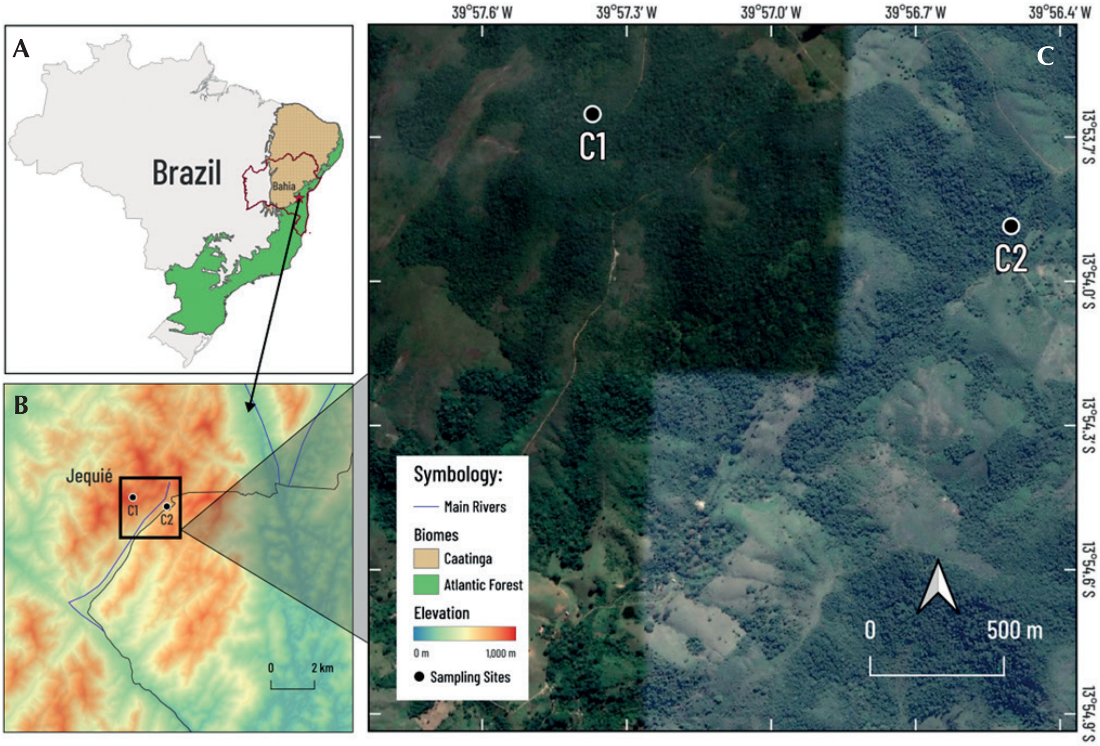

Figure 1. Location of the study area (red star in A). (**A**) General representation of Brazil and the state of Bahia with the presence of the Caatinga (brown) and Atlantic Forest (green) biomes (Source: Google Earth); sampling sites C1 and C2 in Morro do Mara with altimetry (**B**) and satellite view (**C**).

surrounding landscape, the study site remains one of the few areas in the municipality that is still minimally impacted by anthropogenic activities such as agriculture and livestock farming. Although the regional climate is classified as semi-arid, the area lies in a transitional zone, resulting in climatic variability across its breadth. The site includes both lentic and lotic water bodies, ranging from streams to seasonally flooded areas such as swamps and temporary puddles.

In the study area, we monitored two streams and their surroundings: stream 1 (C1), located at 882 m altitude, and stream 2 (C2), located in a lower area, at 675 m a.s.l. (Figure 1). In both locations, the species was recorded in a previous study (Bastos and Zina 2022). Locality C1, where we observed courtship behavior, has a large amount of leaf litter, large trees, and dense vegetation (Amaryllidaceae, Asteraceae, Cyperaceae, Poaceae, and Bromeliaceae). The stream, which is next to a 2.5 m wide road, has crystal clear water ranging in depth from 10 to 40 cm with a sandy bottom and slow flow. Throughout the study, we monitored a 10m length of the stream, which was 2 m wide. C2 is also located in a forest environment and has dense, arboreal vegetation along its margin (Fabaceae, Rubiaceae, Asteraceae, and Pteridophytes), with the presence of large trees. Along the stretch where observations were made (20 m), the stream has a depth ranging from 20 to 80 cm, a maximum width of 6 m, a sandy and rocky bed, and slow flow.

During the study period, field-measured temperatures ranged from 17 to 24°C. The coldest months of the year were May to September, during which the average monthly rainfall was lower, ranging from 32 to 50 mm precipitation. This rainfall data is based on a ten-year average obtained from Proclima/CPTEC/INPE (2021).

Data Collection

We visited the sampling sites 10 times from February 2020 to September 2021 (not continuously), with one or two visits per month. The field observations started around 18:00 h, ending when vocalizations decreased (between 22:00 and 23:00 h), totaling about 60 hours of observation. To locate individuals, we used visual and acoustic cues at breeding sites (see Heyer *et al.* 1994). For behavioral observations, we observed focal animals (e.g., Altman 1973, Lehner 1996) using flashlights at low intensity to reduce the amount of light and possible interference in the behavior of the observed individuals.

We collected the following information during sampling: characteristics of vocalization and reproductive sites (height and type of vegetation used as perch), vocalization shift, number of calling males, the distance between calling males, air temperature, and relative humidity. We measured the height of vocalization sites with a tape if it was under 2 m, or we visually estimated heights greater than 2 m. We sacrificed two individuals using 5% lidocaine, which were later fixed in 10% formaldehyde and preserved in 70% alcohol. These specimens were placed in the zoological collection of the Universidade Estadual do Sudoeste da Bahia (UESB)-Campus Jequié (catalog numbers: MHNJCH 00001644; MHNJCH 00001645). A clutch of eggs was collected, fixed, and preserved (cataloged in the zoological collection of UESB-Jequié MHNJCH 00001642), and the eggs were counted using a magnifying glass. We measured the snout–vent length (SVL) of individuals collected in the field using a caliper with an accuracy of 0.1 mm.

Analysis of Calls

Advertisement calls of *B. capra* were obtained using a Marantz® digital recorder (model PMD660) with a coupled Sennheiser® directional microphone (model ME66). Calls were analyzed using software Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics, 2024) with window type = Hann, window size = 12 samples, 5 AVE rate 44.1 kHz, 16-bit precision, and overlap = 75%. Air temperature and relative humidity were measured immediately after all recordings using a thermohygrometer (Incoterm). The recorded calls were categorized according to the social context following Toledo *et al.* (2014). We recorded the time when the vocalization started until it ended or until the point when the interval between calls became sufficiently long, indicating the impending cessation of activity. We analyzed the following temporal and spectral parameters of the calls: pulse number (PN), interval between pulses (IP), note duration (ND), interval between notes (IN), dominant frequency (DF), and frequency amplitude (FA). The units of analysis for vocalizations follow the terminology proposed by Napoli and Pimenta (2009) for *B. capra.* The beginning and end of each note were determined from oscillograms.

Statistical Analysis

To determine whether reproductive activity is seasonal, we analyzed differences in the number of vocalizing males across seasons (dry and rainy). Months were grouped by season, and an Analysis of Variance (ANOVA) was performed. To assess the effect of climatic variables on the number of vocalizing males, we used a generalized linear mixed model (GLMM) with a Bayesian approach, incorporating the Markov Chain Monte Carlo (MCMC) method. This method allows us to incorporate the seasonal effect in the model as a random effect, thereby removing the influence of the collection station from the regression parameters we wish to

Table 1. Regression coefficients from the Generalized Linear Model (GLM) assessing the effect of climatic variables (temperature and relative humidity) on the number of vocalizing males. PM: Posterior Mean; L95%-CI: Lower Credible Interval; U95%-CI: Upper Credible Interval; ES: Effective Sample Size; pMCMC: Bayesian p-value; * Significant at $p < 0.05$; ** Significant at $p < 0.01$.

	PM	L95%-CI	U95%-CI	ES	pMCMC
(Intercept)	1.884.359	520.199	3.167.845	1914	0.0144 *
Temperature (°C)	-35.265	-67.010	-4.202	1250	0.0368 *
Relative air humidity (%)	-14.461	-24.036	-5.599	1250	0.0080 **

estimate (Sun *et al*. 2000). The analysis was performed with 1,500,000 interactions, 250,000 interactions as burn-in and a sampling interval (thinning) at every 1000 interactions. Non-informative priors were assumed for the variance and covariance matrices of fixed (V), random (G), and residuals (R) effects. Descriptive statistics, model summaries, and convergence tests were performed from a sample of 1250 chains obtained using software R (version 4.3). All parameters converged (Geweke, $p > 0.05$), and the chains passed the seasonality test.

Results

Habitat Use and Seasonality

We only recorded *Bokermannohyla capra* in lotic environments at MM; therefore, these environments were selected for monitoring and sampling. We recorded males of *B. capra* calling from the ground and, more commonly, perched on branches in vegetation along the margins of streams. The highest calling perches (6 m) were recorded in the warmest and wettest months of our sampling (Figure 2). During the driest and coldest months of the year, we observed the use of epiphytic bromeliads as vocalization sites at the lowest elevations (3–4 m). Because bromeliads were at a minimum height of 3 m, it was not possible to observe which portion of these plants were used for calling activity.

Individuals called at night, with a peak between 19:00 and 21:00 h. The number of frogs calling at the same time ranged from one to nine individuals (Figure 3), and the distance between males varied from 3 to 7 m. Males of *B. capra* called throughout the study period; however, two peaks occurred in the number of individuals, one in February 2020 (nine individuals), a rainy month, and the other in September 2021 (eight individuals), a dry month. In the GLMM analyses all parameters were significant (Table 1). The negative slope for temperature indicates that the number of observed individuals decreases with the increase in temperature (Figure 4). Population density did not differ between seasons (Table 2), and it appears that there is no relationship between the highest records and the measured factors. Although the model suggests a decrease in the number of individuals observed as temperature and humidity increase, the empirical data suggest the opposite, with the highest number of records in the hot and rainy season.

Table 2. Descriptive statistics of male incidence and climatic variables collected in Morro do Mara, municipality of Jequié, state of Bahia. The values represent the mean ± standard deviation. ^{a}Treatments followed by the same letter do not differ statistically. Numbers of males (GLM, p = 0.303). Temperature (ANOVA, p = 0.08). Humidity (ANOVA, p = 0.353).

	Rainy season	Dry season
Number of males	4 ± 3.082^a	2.8 ± 3.033^a
Temperature (°C)	21.74 ± 2.173^a	18.86 ± 2.408^a
Relative Air Humidity (%)	73 ± 10.44^a	79.2 ± 9.418^a

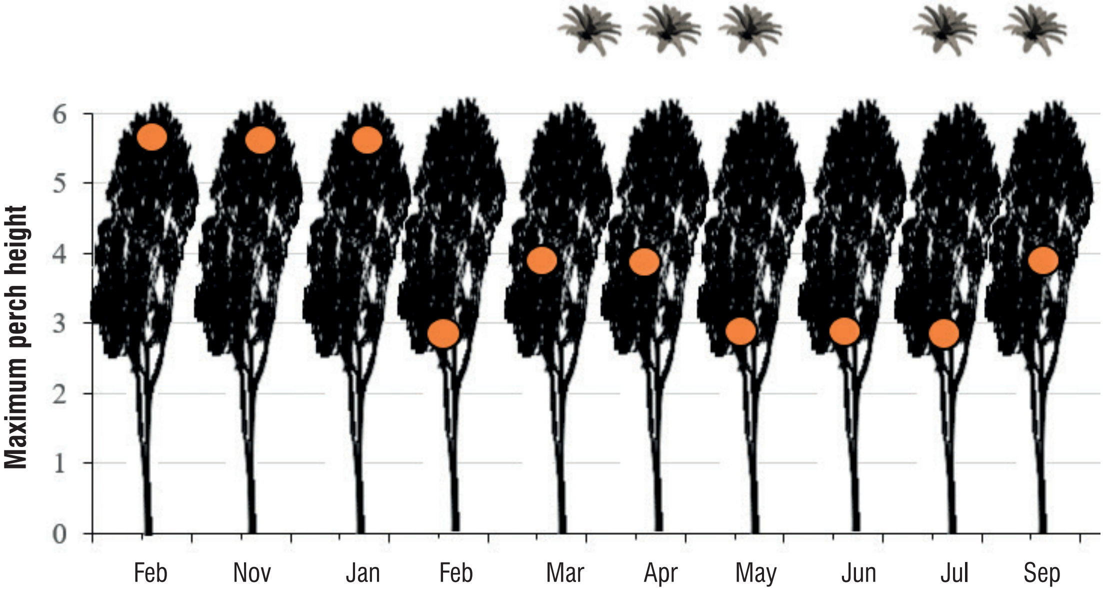

Figure 2. Perch height and use of bromeliads by *Bokermannohyla* from February 2020 to September 2021 in Morro do Mara, Jequié municipality, state of Bahia, Brazil.

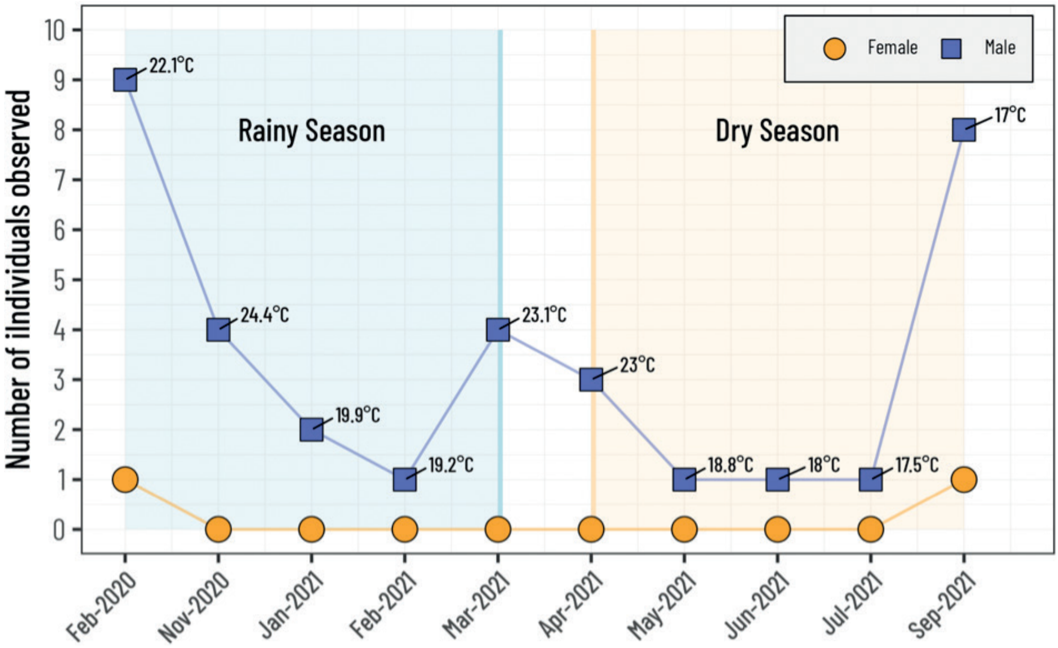

Figure 3. Vocalizing males and females observed in the stream studied from February 2020 to September 2021. Morro do Mara, municipality of Jequié, state of Bahia, Brazil.

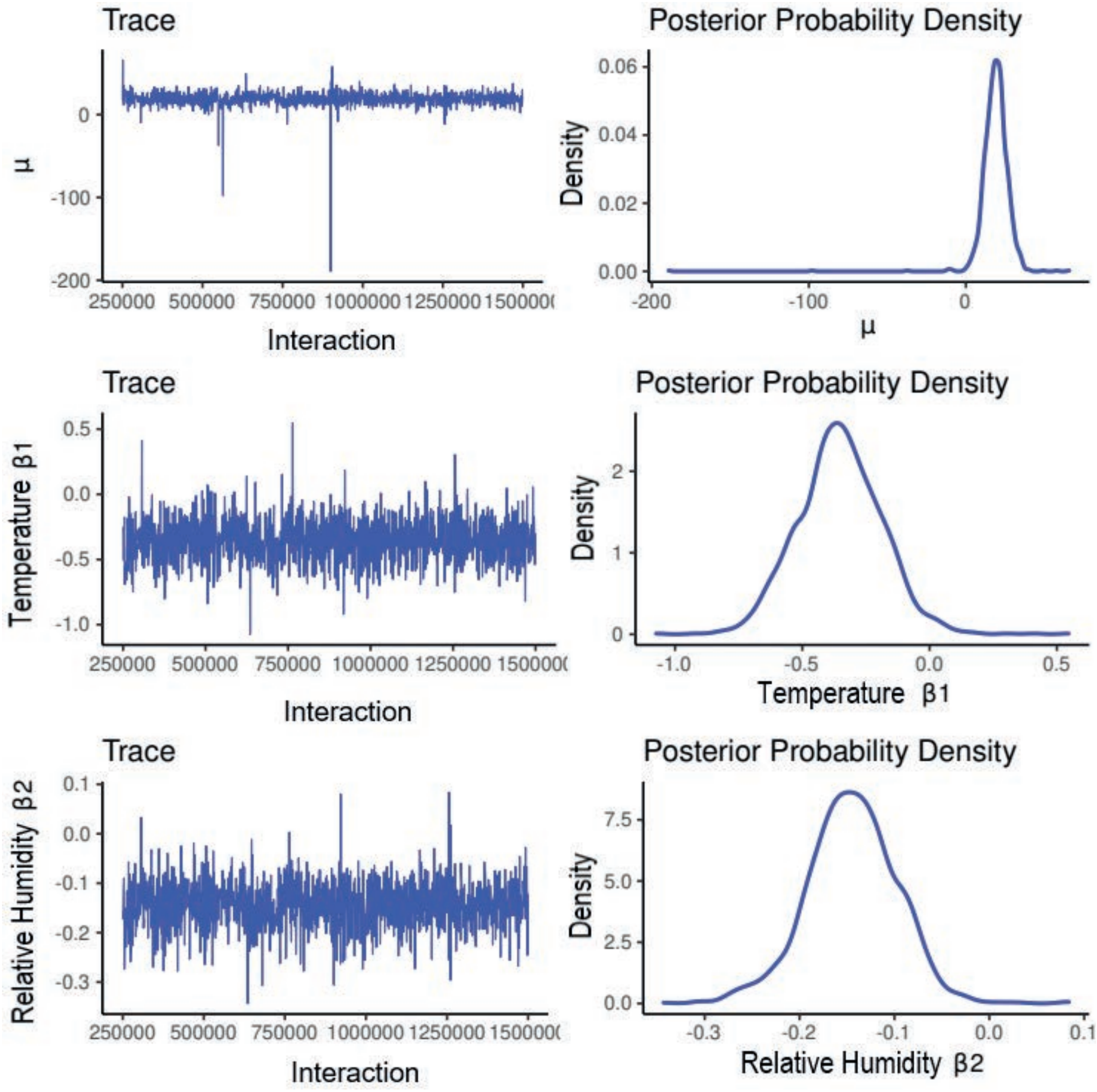

Figure 4. Temperature and humidity parameters indicating that the number of individuals observed decreases with the increase in the magnitude of the two variables. Data collected in Morro do Mara (MM), 20 km from the municipality of Jequié, state of Bahia, Brazil.

Reproductive Behaviors

We observed three courtship interactions (September 2018, February 2020, and September 2021), all of which occurred during nights without rain. The first courtship interaction took place before the sampling period of this study. Only one of the three observed courtship interactions was monitored from the beginning, but this event was unfortunately interrupted by the fall of a tree branch, so it was not carried out until the end.

The other two courtship interactions were observed during their course, one of them being observed to the end. Due to the small sample size, it was not possible to evaluate or even measure the possible selection criteria used by females. All courtship interactions were observed in stream C1, close to the male's vocalization site. In both sampling sites (C1 and C2), we observed tadpoles of *B. capra* attached to rocks and foraging under them. We did not observe aggregation of these tadpoles; only one or two were observed per visit.

The sequence of reproductive behaviors observed during the monitored events can be summarized as follows: at first, the male emitted the advertisement call, with the female perched on a branch close to the male's vocalization site. The male called for a maximum of 50 minutes and then descended from the calling site (bromeliad or tree) and moved closer to the female, positioning himself on the same branch (Figure 5A). After remaining in this position, the male jumped to a smaller branch closer to the ground. The female remained in the same position for a maximum of 40 min, thereafter jumping onto the branch with the male and facing him with her body extending forward. (Figure 5B). A few minutes later, the male jumped to the ground and walked toward the stream (Figure 5C), while being followed by the female (see Figure 5D). Subsequently, the male moved toward a small artificial puddle created by a cattle footprint (approximately 15 cm in diameter and 10 cm in depth) near the stream. At the puddle, the male emitted what we considered a courtship call (an unrecorded vocalization but sounding similar to the advertisement call). The male was immediately followed by the female. The male grabbed the back of the female, positioned himself in the opposite direction to her, and turned three times on the female (Figure 5E). Finally, the male held the female below the axilla, resting his legs over the female's legs, and engaged in axillary amplexus (Figure 5F). After oviposition, the female made quick movements with her legs to arrange the eggs. Amplexus lasted a maximum of 10 minutes. After oviposition, the female and the male separated, neither remaining at the spawning site. The female exited the spawning site first. We collected the egg mass and counted a total of 290 eggs. The eggs are pigmented at the animal pole, exhibiting a whitish cream color at the vegetative pole, each surrounded by a gelatinous capsule.

Courtship events were observed at the same time as territorial and acoustic interactions between males were also recorded. Both occurred on days with the highest density of vocalizing males ($N = 11$, $N = 9$, and $N = 8$, respectively, September 2018, February 2020, and September 2021).

Vocalizations

Calls of three individuals were recorded, totaling 20 analyzed calls. The majority of the analyzed recordings seemed to be the "type B" advertisement call, with rare emissions of call types A and D (sensu Napoli and Pimenta 2009). The advertisement call (Figure 6) is composed of multi-pulsed notes with 3–4 groups of pulses. The calls were emitted both in the presence and absence of females and were characterized as follows: pulse number of 56━111 pulses; interval between pulses of 0.009–0.08 s; note duration of 0.353–0.513 s; interval between notes of 0.825–5.554 s; dominant frequency 1.38–1.72kHz, and frequency amplitude of 0.89–2.52kHz.

In the field, we observed acoustic interactions between neighboring males that were up to 50 cm apart. Although we did not record these interactions, both males emitted calls similar to the advertisement call of *B. capra,* but shorter in duration. The interaction did not escalate into physical combat, and one of the males ceased calling. Males did not respond acoustically to playback experiments, but one individual did move toward the recorder.

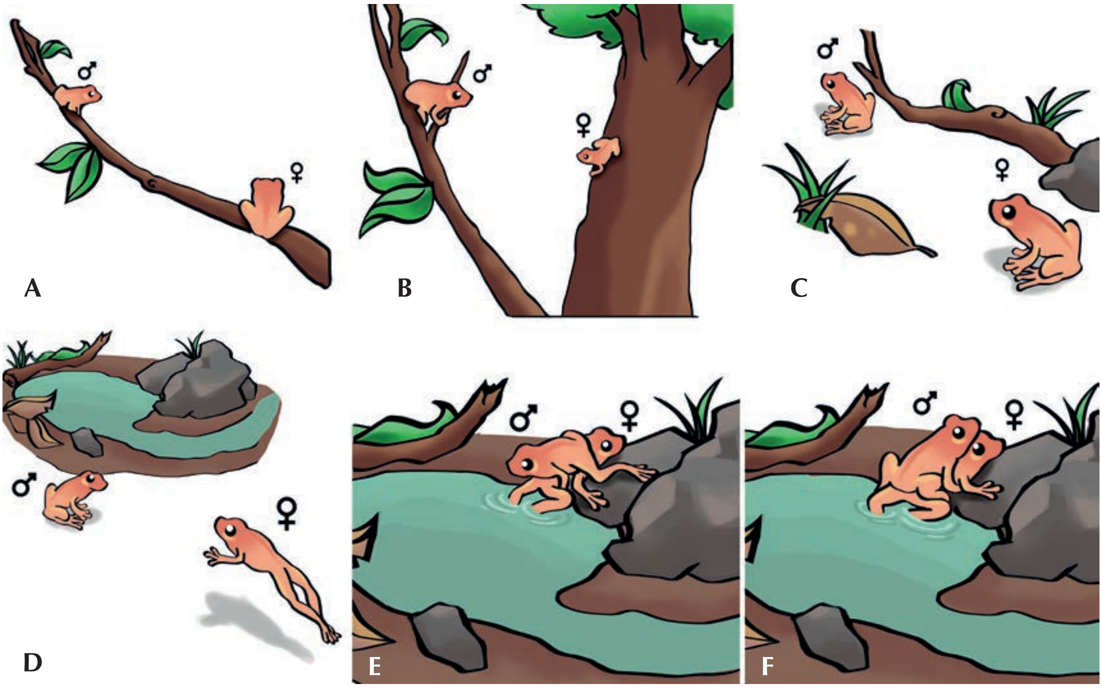

Figure 5. Reproductive behavior of *Bokermannohyla capra* recorded in Morro do Mara (MM), 20 km from the municipality of Jequié, State of Bahia, between 2020 and 2021. (**A**) The male emitted a courtship call directed toward the female; (**B**) the female faced the male, lifting and extending her body forward; (**C**) the male walked toward the water body; (**D**) the male being followed by the female toward the water body; (**E**) the male positioned himself in the opposite direction of the female and rotated three times over the female; (**F**) male entered axillary amplexus with the female.

Discussion

Habitat Use and Seasonality

We observed *B. capra* using vegetation along the margins of lotic environments as a shelter and a vocalization site, following the pattern recognized for most species of *Bokermannohyla* (Haddad and Prado 2005, Haddad *et al.* 2013, Malagoli *et al.* 2021). In addition, *B. capra* used epiphytic bromeliads as vocalization sites. Due to the height of the bromeliads, we were unable to observe *B. capra* using this phytotelm as an oviposition site, thus it can not be classified as a bromeliad-dwelling (sensu Peixoto 1995). However, we do not rule out this possibility, because species that reproduce in bromeliads typically inhabit the arboreal stratum and deposit fewer eggs compared to those that use other aquatic environments (Alves-Silva and Silva 2009), both traits observed for *B. capra* in this study. The use of bromeliads is known for several species within Cophomantini, including other representatives of the genus *Bokermannohyla,* such as *Bokermannohyla astartea* (Bokermann, 1967) (Malagoli *et al.* 2021), which may be considered as an eventual bromeligenous species (sensu Peixoto 1995) (see Malagoli *et al.* 2021). The continuum between using this resource initially as a vocalization site

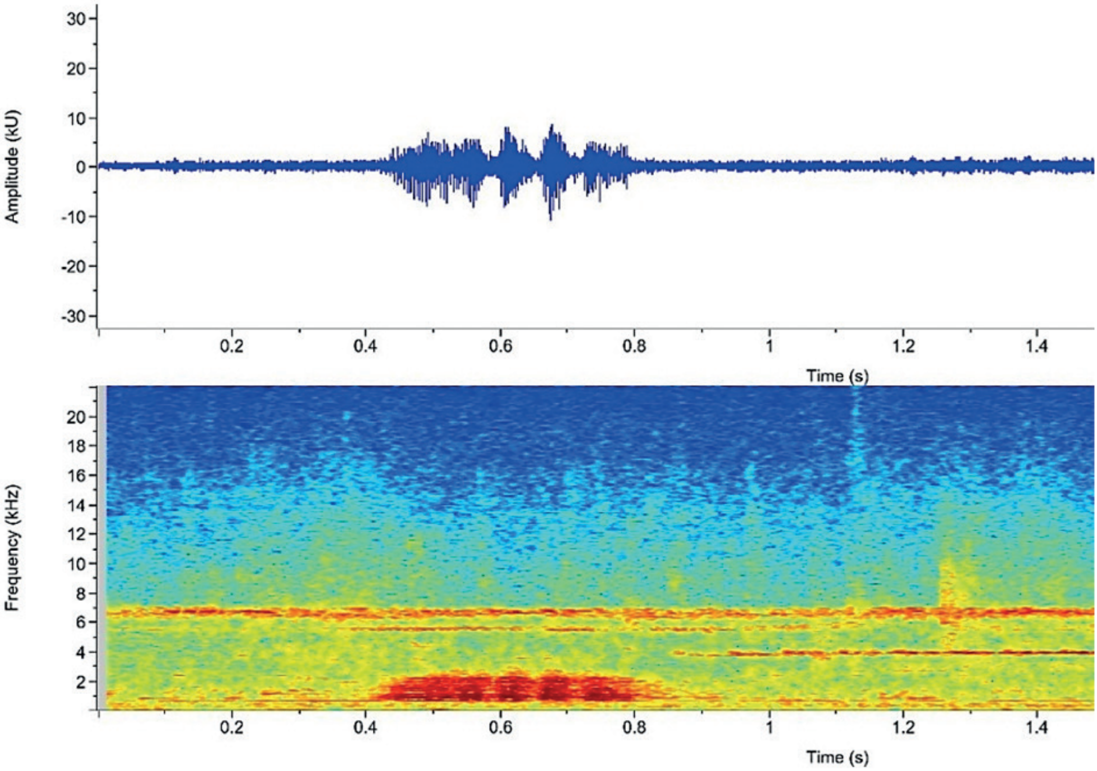

Figure 6. Oscillogram and spectrogram of the advertisement call of *Bokermannohyla capra* recorded in C1, Morro do Mara (MM), municipality of Jequié, state of Bahia, Brazil. Oscillogram—Y Axis: Amplitude (KU); X Axis: Time (s). Spectrogram—Y Axis: Frequency (KHz); X Axis: Time (s). Recorded at a temperature of 22.1°C.

and then as an oviposition site, observed in many anuran species, is a subject to be explored in further studies (Peixoto 1995, Lantyer-Silva *et al.* 2014).

The seasonal use of phytotelms by *B. capra* does not appear to be related to competitor density or rainfall. It may be associated with using the phytotelm as shelter against predators (Ferreira and Faria 2021). We observed greater bromeliad use during the cold season when reproductive activity decreases, making vocalizing males more vulnerable to acoustically oriented predators. Individuals may vocalize inside bromeliads during periods of low activity to reduce predation risk, since only a few males are exposed while vocalizing (Ferreira and Faria 2021).

Because we recorded low male density for *B. capra,* the higher perch heights observed during the warmer and wetter period do not seem to be related to competition for calling sites, which would force some males to choose alternative perches (see Mello *et al.* 2018). Instead, we suggest higher perch heights are associated with increased stream water flow in the wet period, which raises external noise levels. The higher vocalization sites could represent a way to escape from the noisy environment produced by the fast waters that can disturb the emission of the calls, as observed for *Aplastodiscus arildae*

(Cruz and Peixoto, 1987) (see Zina and Haddad 2007). Further studies are needed to confirm the reasons for the change in microhabitat use.

The use of vocalizations to attract females is a typical behavior of prolonged breeding species, with long periods of calling activity and territoriality (Wells 1977). *Bokermannohyla capra* has a reproductive period of over three months, a fact corroborated by Bastos and Zina (2022) in a study of an anuran community in MM. Other observations may indicate that *B. capra* has a prolonged reproductive pattern (sensu Wells 1977), such as the use of permanent environments to reproduce. Reproduction in lotic environments enables longer reproductive periods because oviposition sites are available throughout the year and individuals are not dependent on seasonal climatic aspects (e.g., rainfall and humidity) (Zina and Haddad 2006).

Although we did not observe a marked difference in activity due to season, climatic variables still impact the reproductive activity of *B. capra,* since air temperature and humidity affect the number of males. Long-term studies of monitored populations could provide insight into seasonal patterns and identify the metrics (or combinations of metrics) that influence these patterns.

Reproductive Behavior

According to the classification described by Haddad and Prado (2005) and the data collected, we can classify *B. capra* as having reproductive mode 2 because this species deposits aquatic eggs in cavities not constructed by males in lotic waters where exotrophic tadpoles develop. *Bokermannohyla capra* has axillary amplexus, which also occurs in most anuran species (Wells 2007).

The number of eggs (290) in one clutch of *B. capra* we collected was similar to one that was deposited ex situ in January 2025 (389 eggs). *Bokermannohyla capra* has a small clutch when compared with other species of *Bokermannohyla,* such as *B. circumdata* (Cope, 1871) (1176), *B. napolii* Carvalho, Giaretta, and Magrini, 2012 (637) (Mongin *et al.* 2013), and *B. luctuosa* (Pombal and Haddad, 1993) (396 and 613) (Pombal and Haddad 1993). The reasons behind these interspecific differences remain to be studied, but they may result from environmental use or species-specific factors. Clutch size may be a flexible trait influenced by environmental conditions and natural history, allowing a rapid response to random and unpredictable environmental factors (Liao *et al*. 2014). It may also serve as a valuable trait for phylogenetic and natural history studies that highlight differences within a genus (Gomez-Mestre *et al*. 2012). If the deposition of a few eggs at multiple times during the breeding season is possible (especially for species with a long breeding season) (Prado and Haddad 2005), parental fitness may increase (Wells 2007) and may become a fixed population trait and ultimately a species characteristic. Studies analyzing the factors driving clutch size variation are needed.

Complex courtship behaviors can be found in diverse hylid genera (e.g., Haddad and Sawaya 2000, Zina and Haddad 2007). *Bokermannohyla* is not an exception and is similar to other stream-dwellers such as *B. alvaregai* (Bokermann, 1956)*, B. ibitiguara, B. luctuosa,* and *B. nanuzae* (Bokermann and Sazima, 1973)*,* in which elaborate courtship behavior includes vocalizations, tactile contact, and visual signals, as well as males leading females to oviposition site (see Zornosa-Torres and Toledo 2019). The courtship behavior of *B. capra* encompasses visual stimuli (e.g., female body elevation), tactile stimulation (e.g., male positioning at the end of the courtship), and acoustic stimuli (e.g., advertisement and courtship calls).

The complexity of courtship repertoires tends to increase in species breeding in noisy environments such as lotic water bodies, as seen in Hylodidae (de Sá *et al.* 2016). The use of stimuli other than vocalizations may be crucial for species living in noisy environments and ovipositing far from their calling sites (see Zina and Haddad 2007). In such environments, males must guide females to oviposition sites while overcoming environmental acoustic interference, which requires alternative communication strategies to ensure message clarity and effective reproduction.

Vocalizations

Acoustic communication is the primary mode of intraspecific communication among frogs (Wells 2007, Forti *et al.* 2019). By calling, males try to stand out from competitors in order to attract females and fertilize eggs, ensuring gene transmission to the next generation (Duellman and Trueb 1994, Garcia *et al.* 2001, Forti *et al.* 2019). For each social context, frog calls have variable structure (Narins *et al.* 2006). *Bokermannohyla capra* calls are an example of this variable structure because different types of notes seem to be emitted in specific social contexts.

The advertisement call documented in this study differs from that described by Napoli and Pimenta (2009) for populations from Amargosa, Wenceslau Guimarães, Camamu, and Valença, all located in the Brazilian state of Bahia. In our recordings, type B notes were more frequently emitted, whereas the aforementioned authors reported type A notes as predominant. A comparison between our type B notes and those described by Napoli and Pimenta (2009) revealed similarities in the number of pulses and frequency range (0.99–2.84 kHz and 0.89–2.52 kHz, respectively). Nonetheless, subtle differences were observed: the notes in our dataset exhibit longer duration (0.22–0.39 s vs. 0.35–0.51 s), longer inter-note intervals (0.16–3.72 s vs. 0.82–5.55 s), and a lower dominant frequency (1.80–2.32 kHz vs. 1.38–1.72 kHz).

The differences observed in the advertisement call of *Bokermannohyla capra* from MM when compared to the description provided by Napoli and Pimenta (2009) may reflect a population-specific trait. Such variation could result from isolation by distance and distinct historical selective pressures acting on different populations (Rebouças *et al*. 2020), including differences in environmental conditions and habitat characteristics across the range of the species.

The information provided here on the natural history of *B. capra* reinforces how deficient our understanding of the natural history of species of *Bokermannohyla* treefrogs is. Furthermore, it exemplifies the necessity of studies on natural history. The lack of basic information on reproductive biology makes it challenging to formulate conservation strategies for some species, especially for those with restricted geographic distributions and low densities. The study site (Morro do Mara) is home to a great richness and diversity of amphibians (Bastos and Zina 2022), including endangered species. Its protection is crucial, as ongoing research continues to reveal diverse reproductive behaviors driven by its amphibian richness.

Acknowledgments

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Trophic and spatial ecology of *Pristimantis relictus* (Anura: Strabomantidae) in Atlantic Forest remnants of northeastern Brazil

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Abstract

Trophic and spatial ecology of *Pristimantis relictus* (Anura: Strabomantidae) in Atlantic Forest remnants of northeastern Brazil. Natural history studies offer insights into the behavior and interactions of species in various environments, which are crucial for conservation and preservation. *Pristimantis relictus* is a newly described Robber Frog endemic to the isolated mountains of Ceará state, northeast Brazil. Limited information on its ecology is currently available. Herein we describe the feeding habits and microhabitat use of this species from three mountains in Ceará state. We also performed a literature review of the prey items consumed by other species in the genus *Pristimantis* in the Americas. In general, *P. relictus* is a generalist in terms of microhabitat use, foraging in herbaceous plants, on the ground, trees, and shrubs, and for diet, consuming a wide range of prey groups, with beetles and larvae the most common prey. This plasticity of dietary and microhabitat use may be crucial for the persistence and geographic distribution of this species, including their response to environmental changes. The large variety of prey categories utilized by species of *Pristimantis* suggests a generalist diet that may reflect prey diversity in the environment.

Keywords: Amphibians, Diet composition, Food network, Microhabitat use.

Resumo

Ecologia trófica e espacial de *Pristimantis relictus* (Anura: Strabomantidae) em remanescentes de Mata Atlântica do nordeste do Brasil. Estudos de história natural oferecem informações sobre o comportamento e as interações de espécies em vários ambientes, que são cruciais para a conservação

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e preservação. *Pristimantis relictus* é uma perereca recém-descrita, endêmica das montanhas isoladas do estado do Ceará, nordeste do Brasil. Informações limitadas sobre sua ecologia estão disponíveis atualmente. Aqui, descrevemos os hábitos alimentares e o uso de micro-hábitats dessa espécie em três montanhas no estado do Ceará. Também realizamos uma revisão da literatura sobre as presas consumidas por outras espécies do gênero *Pristimantis* nas Américas. De modo geral, *P. relictus* é generalista em termos de uso de microhabitat, forrageando em plantas herbáceas, no solo, árvores e arbustos, e em termos de dieta, consumindo uma ampla gama de grupos de presas, com besouros e larvas sendo as presas mais comuns. Essa plasticidade do uso dietético e de microhabitat pode ser crucial para a persistência e a distribuição geográfica dessa espécie, incluindo sua resposta a mudanças ambientais. A grande variedade de categorias de presas utilizadas pelas espécies de *Pristimantis* sugere uma dieta generalista que pode refletir a diversidade de presas no ambiente.

Palavras-chave: Anfíbios, Composição da dieta, Rede alimentar, Uso de micro-hábitats.

Introduction

Amphibians are one of the most diverse groups of vertebrates, inhabiting a wide range of microhabitats around the world (Frost 2024). Despite their high diversity, some areas of the world remain poorly explored. Amphibians are at a greater risk of extinction than any other vertebrate group (Wake and Koo 2018), mainly due to human impacts (Sodhi *et al.* 2008). Natural history studies provide a good tool to understand how these frogs interact with others and with the environment and provide a starting point for studies aiming at conservation and preservation (Solé and Rödder 2010, Michaels *et al.* 2014), although relatively little is known about the natural history of most anurans.

Feeding habits form a significant aspect of the natural history of a species (Duellman and Trueb 1994). In amphibian populations, diet composition varies considerably, depending on the species, habitat, and environmental conditions, among other variables (Toft 1981, Duellman and Trueb 1994, Blanco-Torres *et al.* 2020). Some species, such as *Elachistocleis bicolor* (Guérin-Méneville, 1838), are ant specialists (López *et al.* 2007), and others feed on fruits, flowers, and nectar, such as *Xenohyla truncata* (Izecksohn, 1959) (Silva and Britto-Pereira 2006, Oliveira-Nogueira *et al.* 2023), but most anurans are carnivores, generalists, and opportunistic predators (Toft 1981). These animals are components of different food webs, sometimes as predators (Ceron *et al.* 2019) and sometimes as prey (Brown and Shine 2002).

The genus *Pristimantis* Jiménez de la Espada, 1870, comprised of 612 species, is one of the most diverse groups of terrestrial frogs. They occur from Honduras eastward through Central America to Colombia and Ecuador to Peru, Bolivia, northern Argentina, the Amazonian and Atlantic Forests of Brazil, and the Guianas; Trinidad and Tobago; and Grenada, Lesser Antilles (Frost 2024). In addition, some populations of these direct-developing frogs also occur in rainforest enclaves in the Caatinga biome (Roberto *et al.* 2022). Despite this high species diversity and some natural history studies (e.g., Arroyo *et al.* 2008, Hutter *et al.* 2016, Alves-dos-Santos *et al.* 2021), there is a lack of information concerning the ecological characteristics of some species of *Pristimantis.*

Species of *Pristimantis* are diet generalists and feed opportunistically on dipterans, arachnids, collembolans, coleopterans, and hymenopterans, among other invertebrates (García-R *et al.* 2015, Arroyo *et al.* 2008). In Brazil, *P. paulodutrai* (Bokermann, 1975) (Alves-dos-Santos *et al.* 2021) and *P. ramagii* (Boulenger, 1888) (Leite-Filho *et al.* 2017, Caldas *et al.* 2019) were generalists with an opportunistic diet. Relatively little is known about other species, including the newly described *Pristimantis relictus* Roberto, Loebmann, Lyra, Haddad, and Ávila, 2022. Herein we describe its microhabitat and diet composition and test the hypothesis that larger frogs tend to eat larger prey items. We also provide a literature review of the most frequently consumed prey items by species of *Pristimantis* in the Americas.

Materials and Methods

Study Area

Our study area included three mountains located in the state of Ceará (Figure 1), northeastern Brazil: Baturité (-4.20547° S, -38.96047° W; datum WGS84; elevation: 900 m), Maranguape (-3.90464° S, -38.72051° W; datum WGS84; elevation: 920 m), and Meruoca (3.54769° S, -40.4486° W; datum WGS84; elevation: 800 m). These mountain chains have rainforest enclaves called "Brejos de altitude" in the midst of the xerophytic vegetation of the Caatinga (Andrade-Lima 1982). They receive high and evenly distributed rainfall (Medeiros and Cestaro 2019) and support three main vegetation types: humid forests found at high elevations, dry forests at mid-elevations, and caatinga in the lowlands (Moro *et al.* 2015).

Anuran Sampling

We manually collected adult individuals of *P. relictus* (Figure 2) using visual and auditory searches (Heyer *et al.* 1994) at night (17:30 to 00:00 h) from February to May 2019.We collected for 15 days in each mountain, totaling 45 days of sampling. For each anuran, we recorded the substrate on which it was found (herbaceous plants, on the ground, trees, and shrubs). All collected individuals were euthanized (Permit: ICMBio 72384, process: 29613) following resolution No. 37 (CONCEA 2018), fixed in 10% formalin, and preserved in 70% alcohol. Voucher specimens are held at the Núcleo Regional de Ofiologia of the Universidade Federal do Ceará, Fortaleza, Brazil (CHUFC

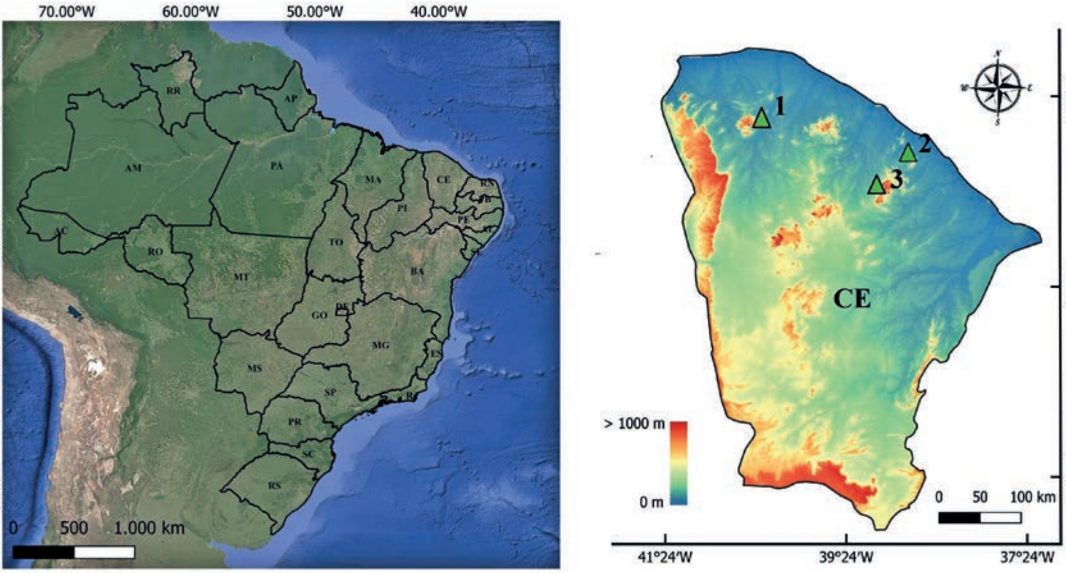

Figure 1. Schematic map of the mountains of Meruoca (1), Maranguape (2), and Baturité (3), where individuals of *Pristimantis relictus* were collected. Green triangles represent the sampled mountains in Ceará state (CE), northeast Brazil.

11726–11732, 12025, 12026, 12029–12033, 12035, 12039–12042, 12049, 12051, 12052, 12833, 12838, 12840, 12843, 12844, 12847, 12851, 12852, 12854, 12856, 12858, 12859, 12862, and 12864).

Data Analyses

In the laboratory, we measured snout–vent length (SVL) and mouth width with a digital caliper (0.01 mm precision) for each specimen. Using a central incision, we removed and identified stomach contents. Prey items were identified to the lowest taxonomic level using the most recent literature in Brazil (Rafael *et al.* 2024). We measured the length and width of each prey item with a digital caliper (accuracy 0.01 mm) and used the ellipsoid formula $V = (4/3)\,\pi\,(L/2)\,(W/2)^2$, where V = volume, L = length, and W width (Dunham 1983) to calculate prey volume. To investigate the relative contribution of each prey category, we used the Index of Relative Importance [$IRI = (F\% + N\% + V\%)/3$], where $F\%$ is the percentage of occurrence, $N\%$ is the numeric percentage, and V% is the volumetric percentage of each prey item (Powell *et al.* 1990).

We used the Kruskal–Wallis H test to investigate whether the microhabitats used (herbaceous, trees, soil, and shrubs) by *P. relictus* differed between the three mountains studied and used bar plots to show these differences. Regarding diet composition, we used sample-based accumulation curves with 1000 randomizations from an incidence matrix (Gotelli and Colwell 2001) to evaluate whether our sampling methods were satisfactory to estimate the prey composition. The estimators Jackknife 1 and Chao 1 were used to investigate the expected prey richness. Non-parametric Kernel regression test examined how the volume of each prey ingested related to SVL and mouth width.

To compile the most frequently consumed prey items by species of *Pristimantis* in the Americas, we performed literature searches in scientific publications available from seven electronic databases (Google, Google Scholar, PubMed, Scielo, Science Direct, Scopus, and Web of Science) using the following keywords in English and Portuguese: America, Brazil, Craugastoridae, diet composition, *Eleutherodactylus,* feeding habits, Pristimantinae, *Pristimantis,* South America, and Strabomantidae. We constructed a matrix of presence and absence data of prey categories for 29 species of *Pristimantis* distributed in five countries (Brazil, Colombia, Ecuador, Peru, and Venezuela) in South America, and we used a network graph to explore this prey diversity. Statistical analyses were done using the R packages bipartite (Dormann *et al.* 2009), igraph (Csardi and Nepusz 2006) and vegan (Oksanen *et al.* 2016).

Figure 2. Adult male of *Pristimantis relictus* in Mt. Maranguape, Ceará state, northeastern Brazil.

Results

We observed 89 individuals of *P. relictus,* with 34 foraging on herbaceous plants, 33 on the ground, 13 in trees, and nine in shrubs. We did

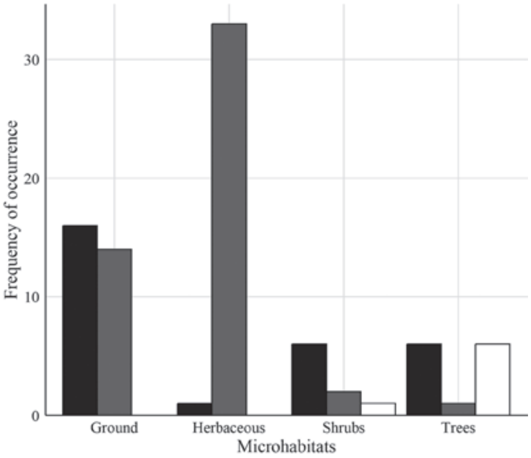

Figure 3. Microhabitats used by *Pristimantis relictus* in rainforest enclaves in Ceará state, northeastern Brazil. White, black, and gray columns represent the mountains of Meruoca, Baturité, and Maranguape, respectively.

not find sufficient evidence to state that the usage of these microhabitats varied significantly among the three mountains (H = 3.6137, P = 0.1642). Despite this result, the individuals collected on Mt. Maranguape were associated more frequently with herbaceous vegetation and on the ground, whereas those collected on Mt. Baturité were more frequently associated with shrubs, and those from Mt. Meruoca were more frequently associated with trees (Figure 3).

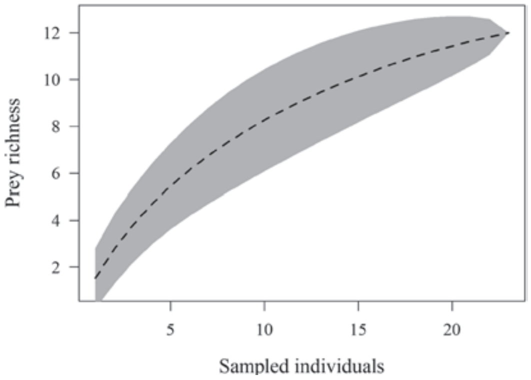

Figure 4. Accumulation curve of the prey items consumed by *Pristimantis relictus* in the studied mountains.

Of the 89 observed individuals, we collected 48 (20 males and 28 females), of which 31 (10 males and 21 females) had full stomachs (65%). Prey diversity was high (12 prey categories), with beetles (IRI = 26.2) and larvae (IRI = 22.3) the most consumed prey items (Table 1). Pieces of plants were found in the stomachs of seven individuals. Our accumulation curve did not reach the asymptote (Figure 4), in which the estimators Jackknife 1 and Chao 1 indicated around four (15.8 ± 1.91) and two (13.9 ± 2.54) more prey categories, respectively, in the diet composition of *P. relictus* at the sampled sites.

We recorded only five prey types in males (Acaridae, Formicidae, Gastropoda, larvae, and plant pieces), whereas 11 prey items were found in female stomachs (Acaridae, Aranae, Blattaria, Coleoptera, Dermaptera, Diptera, Formicidae, Hemiptera, Orthoptera, larvae, and plant pieces). Discrepancies between males and females sampled prohibited intersexual statistical analyses. Neither anuran SVL (R = 0.171, $p > 0.05$) or mouth width (R = 0.307, $p > 0.05$) influenced the choice of prey volume.

Our literature search revealed a generalist pattern of diet composition for the genus *Pristimantis* with 25 prey categories recorded for 29 species distributed in five countries from the Americas. The most frequently consumed items were Coleoptera, Araneae, Diptera, Formicidae, Blattaria, and Orthoptera. Overall, the prey number found in each species ranged from one to 16 (Figure 5, Appendix I).

Discussion

Pristimantis relictus occurs only in the “Brejos de Altitude” of Ceará state in northeastern Brazil. It usually lives in dry and wet forests (Roberto *et al.* 2022).

Some individuals were found near disturbed sites; for example, at Mt. Meruoca, most

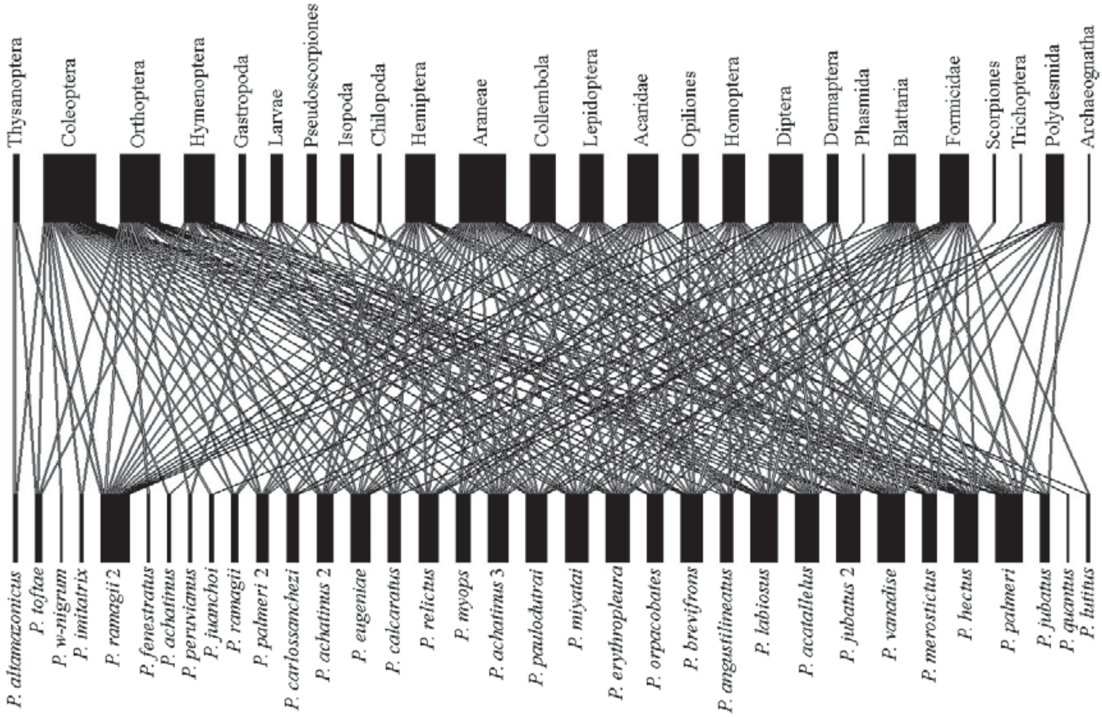

Figure 5. Bipartite network diagram representing predator-prey interactions among different species of the genus *Pristimantis.* The bar width of the predators (left side column, with *Pristimantis* species names) represents niche breadth, and the bar width of the prey (right side column, with arthropod order names) is the level of importance for the diet of the species of *Pristimantis.*

individuals were primarily found in bamboo trees. Although the microhabitat used by *P. relictus* did not vary significantly among the mountains, most individuals at Mt. Baturité were located on the ground. In contrast, at Mt. Maranguape, individuals were predominantly found in herbaceous plants (Figure 2). This plasticity of microhabitat use was observed in other *Pristimantis* species (e.g., Arroyo *et al.* 2008). Direct-developing frogs do not need lentic ponds to reproduce because they have terrestrial eggs and lack a free-living larval stage (Wells 2007, Padial *et al.* 2014), which makes them habitat generalists.

Pristimantis relictus consumed high prey diversity, indicating that it is a generalist predator as observed in most congenerics (e.g., Arroyo *et al.* 2008, García-R *et al.* 2015, Gutiérrez-Cárdenas *et al.* 2016, Alves-dos-Santos *et al.* 2021). Coleopterans and larvae were the most common prey, as observed in other congenerics such as *P. altamazonicus* (Barbour and Dunn, 1921), *P. imitatrix* (Duellman, 1978), *P. toftae* (Duellman, 1978) (e.g., Parmelee 1999), *P. eugeniae* (Lynch and Duellman, 1997) (e.g., Guayasamin *et al.* 2004), and *P. labiosus* (Lynch, Ruiz-Carranza, and Ardila-Robayo, 1994) (e.g., Gutiérrez-Cárdenas *et al.* 2016). Beetles are usually abundant and diverse in the environment, in addition to being difficult to digest. Insect larvae are abundant during the rainy season (Leite-Filho *et al.* 2015),

Table 1. Diet composition of *Pristimantis relictus* from rainforest enclaves in Ceará state, northeastern Brazil. The absolute values and proportion (%) number (*N*), frequency of occurrence (*F*), volume (V) and index of relative importance (IRI) are given. *Prey items consumed exclusively by males, ** prey items consumed exclusively by females.

Prey category	*N*	*N*%	V	V%	*F*	*F*%	IRI
ARACHNIDA							
Acaridae	8	22.2	3.21	0.15	4	14.2	12.2
Aranae**	1	2.78	8.38	0.38	1	3.57	2.24
INSECTA							
Blattaria**	2	5.56	47.5	2.18	2	7.14	4.96
Coleoptera**	10	27.7	407.5	18.6	9	32.1	26.2
Dermaptera**	1	2.78	5.75	0.26	1	3.57	2.20
Diptera**	1	2.78	68.4	3.14	1	3.57	3.16
Formicidae	5	13.8	84.8	3.89	2	7.14	8.31
Gastropoda*	1	2.78	31.6	1.45	1	3.57	2.60
Hemiptera**	2	5.56	217.0	9.95	2	7.14	7.55
Orthoptera**	2	5.56	258.3	11.8	2	7.14	8.18
OTHER ITEMS							
Larvae	3	8.33	1049.8	48.1	3	10.7	22.3

coinciding with the anuran reproductive period. Larvae are a great energy source because of their nutritional content (Marconi *et al.* 2002). Of the 31 individuals, seven had plant pieces in their stomachs, which may be consumed unintentionally during arthropod predation (Hirai and Matsui 1999, Brito *et al.* 2012). The hypothesis of accidental consumption is supported by our findings because plant material was consistently associated with the consumption of other food items and was recorded in a smaller number of individuals. However, consumption of plant material may be beneficial for anurans because it can aid digestion or provide a supplementary source of nutrients and water (Anderson *et al.* 1999). The role of plant material in the diet of *P. relictus* requires further investigation.

We found no relationship between prey volume and SVL or mouth width, as reported for other anurans (e.g., Lima *et al.* 2010, Sabagh *et al.* 2012, Solé *et al.* 2009, 2019), including *P. labiosus* (Gutiérrez-Cárdenas *et al.* 2016). This relationship is typically more apparent when comparing adults and juveniles (Solé and Rödder 2010). The absence of a relationship between anuran size and prey volume may occur when only adult individuals are sampled (Sugai *et al.* 2012), as in the present study. In addition to this discussion, foraging strategy might be the most important driver of prey selection by anurans (Blanco-Torres *et al.* 2020). Our findings suggest that *P. relictus* eats whatever is available in the environment with no prey selectivity; consequently, SVL and mouth width do not appear to be key factors for prey selection.

Although discrepancies between sampled males and females inhibited intersexual analyses, we observed that females consumed a higher prey diversity (10 prey types) compared with males that consumed just five prey types, of which Gastropoda was found only in males. Differences in selectivity of some prey items between males and females may be the result of chance (Sugai *et al.* 2012) given the generalist diet pattern observed. Similarity in other prey items may reflect prey availability in the environment (Sant'Anna *et al.* 2022). Further studies with larger sample sizes are needed to understand intersexual variation in this species.

We observed that species of *Pristimantis* have a generalist diet and consume a high diversity of prey, which is probably a phylogenetically conserved trait (Alves-dos-Santos *et al.* 2021). The various studies were carried out in different locations and periods, which might have contributed to high prey diversity. Among them, *P. ramagii* consumed the highest prey diversity (Leite-Filho *et al.* 2017), whereas *P. quantus* (Lynch, 1998) and *P. w-nigrum* (Boettger, 1892) consumed the lowest, likely because just one individual of each species was examined (García-R *et al.* 2015). In general, the most commonly consumed prey items were Coleoptera, Araneae, Diptera, Formicidae, Blattaria, and Orthoptera, which are frequently found in diets of anurans. This study supports a generalist diet in species of *Pristimantis.*

Pristimantis relictus is a generalist species regarding microhabitat use and feeding habits, which can be linked. As individuals explore different microhabitats, they encounter different prey categories. This plasticity in diet and microhabitat use may be crucial for their persistence and geographic distribution, including their response to environmental changes. In addition, the large variety of prey categories observed in species of *Pristimantis* may suggest a generalist diet, with individuals consuming a high prey diversity wherever they forage. Our study contributes to the understanding of the natural history of the recently described and endemic mountain frog *P. relictus.*

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Appendix I. *The most common prey items in the diet of species of* Pristimantis *in the Americas.*

Species	First Prey	Second Prey	Third Prey	Country	References
P. acatallelus	Diptera	Collembola	Araneae	Colombia	García-R *et al.* 2015
P. achatinus	Araneae	Coleoptera	-	Colombia	García-R *et al.* 2015
P. achatinus 2	Coleoptera	Hymenoptera	Hemiptera	Colombia	Hoyos-Hoyos *et al.* 2012
P. achatinus 3	Coleoptera	Hymenoptera	Araneae	Colombia	Moreno-Barbosa and Hoyos-Hoyos 2014
P. altamazonicus	Thysanoptera	Coleoptera	-	Peru	Parmelee 1999
P. angustilineatus	Diptera	Araneae	Homoptera	Colombia	García-R *et al.* 2015
P. brevifrons	Diptera	Coleoptera	Araneae	Colombia	García-R *et al.* 2015
P. calcaratus	-	-	-	Colombia	García-R *et al.* 2015
P. carlossanchezi	Coleoptera	Araneae	-	Colombia	Arroyo *et al.* 2008
P. erythropleura	Araneae	Formicidae	Diptera	Colombia	García-R *et al.* 2015
P. eugeniae	Coleoptera	Araneae	Hemiptera	Ecuador	Guayasamin *et al.* 2004
P. fenestratus	Orthoptera	-	-	Peru	Parmelee 1999
P. hectus	Diptera	Coleoptera	Araneae	Colombia	García-R *et al.* 2015
P. imitatrix	Coleoptera	Orthoptera	-	Peru	Parmelee 1999
P. juanchoi	Coleoptera	Blattaria	-	Colombia	García-R *et al.* 2015
P. jubatus	Araneae	Hymenoptera	Acari	Colombia	García-R *et al.* 2015
P. jubatus 2	Diptera	Hymenoptera	Araneae	Colombia	García-R *et al.* 2012
P. labiosus	Hymenoptera	Hemiptera	Orthoptera	Colombia	Gutiérrez-Cárdenas *et al.* 2016
P. lutitus	Formicidae	Hemiptera	-	Colombia	Arroyo *et al.* 2008
P. merostictus	Coleoptera	Araneae	Formicidae	Colombia	Arroyo *et al.* 2008
P. miyatai	Collembola	Coleoptera	Formicidae	Colombia	Arroyo *et al.* 2008
P. myops	Diptera	Formicidae	Hymenoptera	Colombia	García-R *et al.* 2015
P. orpacobates	Coleoptera	Araneae	Formicidae	Colombia	García-R *et al.* 2015
P. palmeri	Coleoptera	Diptera	Blattaria	Colombia	García-R *et al.* 2015
P. palmeri 2	Hymenoptera	Hemiptera	Araneae	Colombia	Hoyos-Hoyos *et al.* 2012
P. paulodutrai	Araneae	Isopoda	Formicidae	Brazil	Alves-dos-Santos *et al.* 2021
P. peruvianus	Orthoptera	Larvae	-	Peru	Parmelee 1999
P. quantus	Araneae	-	-	Colombia	García-R *et al.* 2015
P. ramagii	Coleoptera	Hemiptera	Araneae	Brazil	Caldas *et al.* 2019
P. ramagii 2	Hemiptera	Formicidae	Orthoptera	Brazil	Leite-Filho *et al.* 2017
P. relictus	Coleoptera	Larvae	Acaridae	Brazil	**Present study**
P. toftae	Orthoptera	Coleoptera	-	Peru	Parmelee 1999
P. vanadise	Coleoptera	Diptera	Acari	Venezuela	Piñero and Durant 1993
P. w-nigrum	Coleoptera	-	-	Colombia	García-R *et al.* 2015

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SHORT COMMUNICATION

New data on the lack of the occipital scale in *Teira dugesii* (Squamata: Lacertidae) in Madeira and Selvagens Archipelagos

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Keywords: Head scale patterns, Lizards, Madeira Island, Natural history.

Palavras-chave: História natural, Ilha da Madeira, Lagartos, Padrões de folidose cefálica.

Most lacertid species display a consistent pattern of head scale arrangement, with deviations from this pattern being uncommon. Unusual morphological changes in vertebrates can be attributed to varying development rates and heterochronic events (Klingenberg 1998, Hanken 2015). Some authors considered this situation to be dependent on taxonomic status, geographic location, sex, or environment (Clarke 1993, Batterham *et al*. 1996). Deep and open waters act as natural barriers to the gene flow of terrestrial animals, promoting the isolation and differentiation of island populations (Jønsson *et al*. 2017, Mapel *et al*. 2021). In small island populations with few or no predators, genetic diversity is often driven by genetic drift and selection due to competition for limited local resources (Otalora *et al*. 2024). Smaller islands are expected to have lower genetic diversity than larger islands (James *et al*. 2016). Some of the most recurrent examples of morphological or pattern changes occur on small islands (Thorpe and Malhotra 1998, Göltenboth 2006). According to Otalora *et al.* (2024) smaller islands showed a higher number of unique alleles, indicating allele fixation and an ongoing differentiation process.

Teira dugesii (Milne-Edwards, 1829) is an endemic species of the Madeira archipelago and Selvagens Islands, located in the Atlantic Ocean, 700 km from Africa and 900 km from Portugal. Introduced populations were found in the Azores islands (Ulfstrand 1961), Lisbon (Sá-Sousa

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1995), Porto (Ferreira *et al*. 2023) and the Canary Islands (Santos *et al.* 2013). In the last century, a combination of studies proposed the recognition of four subspecies: *Teira dugesii dugesii* (Madeira), *Teira dugesii jogeri* (Bischoff, Osenegg, and Mayer, 1989) (Porto Santo), *Teira dugesii selvagensis* (Bischoff, Osenegg, and Mayer, 1989) (Selvagens) and *Teira dugesii mauli* (Mertens, 1938) (Desertas) (Jesus *et al.* 2009).

Jesus *et al.* (2006) reported four patterns for the occipital region. In that study, a high percentage of *Teira dugesii* lacked the occipital scale Selvagem Pequena Island (Selvagens Islands) and a lower percentage lacked this pattern in Selvagem Grande Island (Selvagens Islands). This pattern was not found elsewhere in the original geographic distribution of the species. In the present study, the same four scale patterns represented in Jesus *et al.* (2006) were recognized: (I) normal, with a normal-sized occipital scale found in most animals; (II) a divided occipital scale; (III) a diminished occipital scale; and (IV) no occipital scale. Another group of scale patterns corresponded to deformations of the occipital region (V).

We analyzed 635 specimens from 41 locations (Figure 1) on Madeira Island, Porto Santo Island, and Selvagem Grande, and added information from 371 specimens used in study by Jesus *et al.* (2006). Specimens were randomly captured using pitfall traps baited with bananas. After examining the occipital scales, they were released back into their natural habitat. Due to the lack of significant differences in proportions of head scale patterns among the collection sites

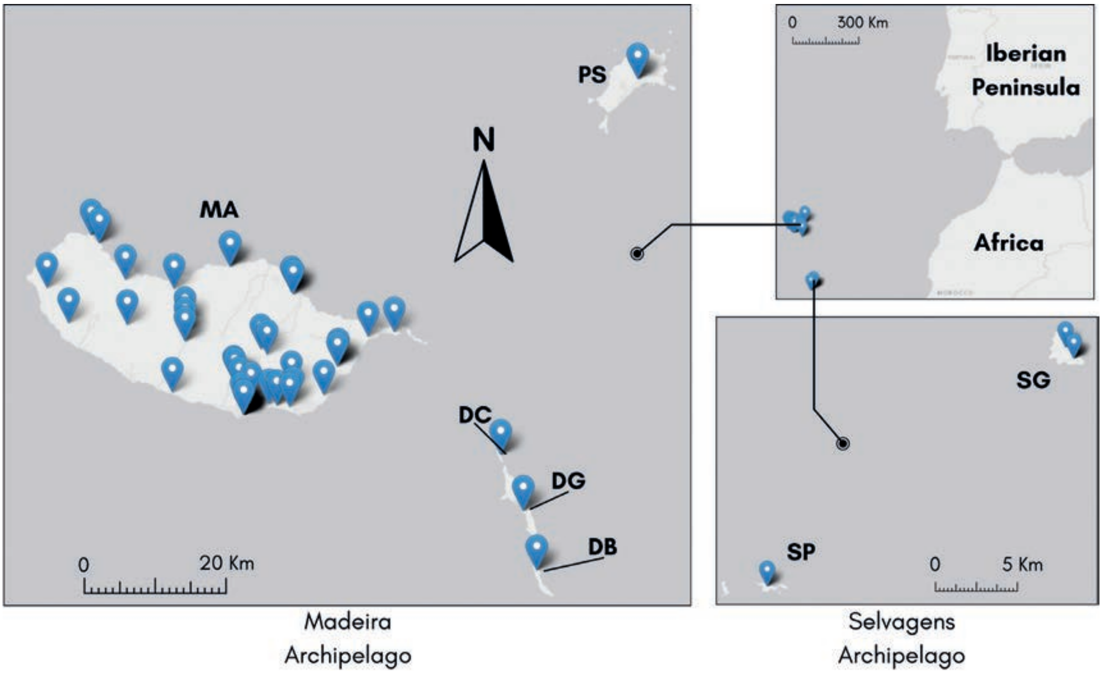

Figure 1. Sampling locations across the Madeira and Selvagens archipelagos, indicating where *Teira dugesii* specimens were collected. Each point represents a sampling site. The abbreviations MA, PS, DC, and DB refer to Madeira, Porto Santo, Ilhéu Chão, Deserta Grande, and Búgio, respectively (all from Madeira archipelago), while SG and SP represent Selvagem Grande and Selvagem Pequena, two islands in the Selvagens archipelago.

within islands, all data from these sites were considered as one sample. The sampling of the 635 specimens occurred between 03 July 2023 and 13 April 2024. Samples from different locations were a minimum distance of 200 m from each other.

A Fisher's Exact Test was used to understand the association between head scalation patterns and sex categories across subspecies. A multinomial logistic regression analysis was conducted to examine the relationship between subspecies and the occurrence of various occipital patterns.

As reported by Jesus *et al.* (2006), Type I is the most common type across all islands. It occurs on all islands of the Madeira archipelago and the Selvagens Islands, while Type II was only absent on Selvagem Pequena. In Porto Santo, as in the study by Jesus *et al.* (2006), only two types of occipital scales, Type I and Type II, were found. Type III was not found in Porto Santo Island and Desertas (Table 1). Specimens of Type IV (Figure 2) were found on all the Selvagens Islands and Madeira Island, contrary to the 2006 study, where it was reported only for the Selvagens islands. On Madeira Island, this type was observed at six different collection points. Only one location on Madeira Island, Funchal, Caminho da Barreira, had all four types of patterns. The Selvagem Grande Island population also includes all types. In the aforementioned study, no location had all four types. In the sample from Selvagem Pequena, Type IV is prevalent, and more than half of the individuals do not have the occipital scale. Curiously, Type IV was not found in males in the

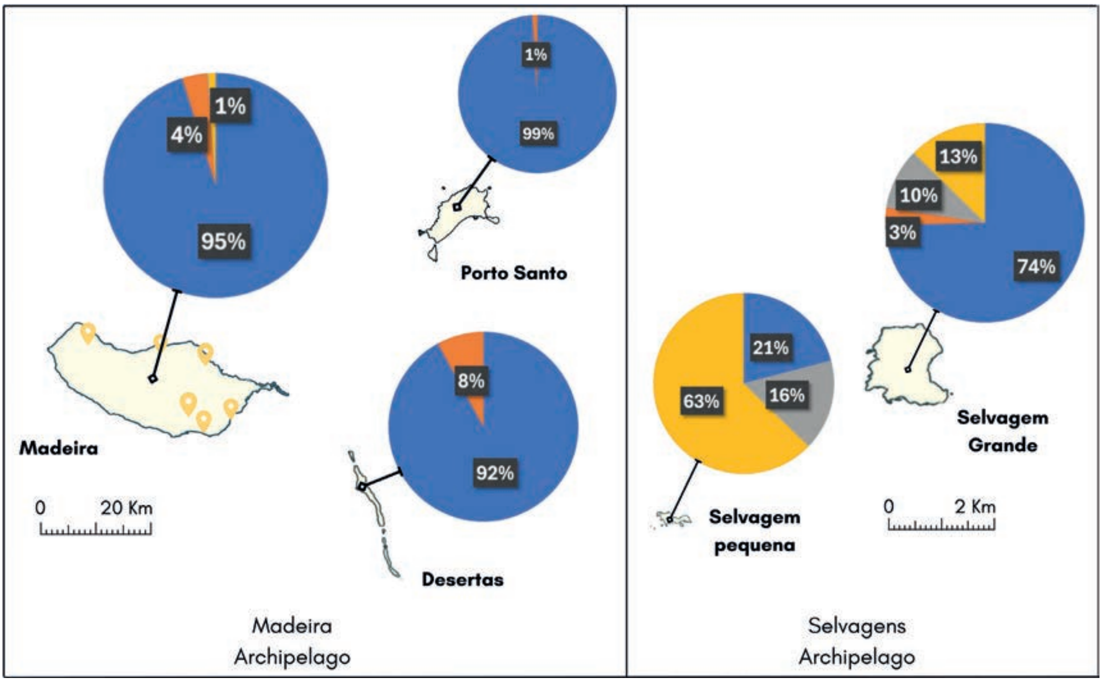

Figure 2. Distribution of the four scale types (excluding deformed scales) across the Madeira and Selvagens archipelago. Pie charts show the percentage frequency of each scale type (Type I: blue; Type II: orange; Type III: gray; Type IV: yellow). The yellow markers on Madeira Island indicate where lizards with scale Type IV were caught.

Table 1. Head scaling pattern types recorded in the geographic range of *Teira dugesii*. Data are presented as % (number). Legend: M, males; F, females; SA, subadults; J, juveniles.

Subspecies	Sex	*N*	**Type I** Occipital scale normal	**Type II** Occipital scale fragmented	**Type III** Occipital scale reduced	**Type IV** Occipital scale absent	**Type V** Deformed occipital scale
Teira dugesii dugesii	M	284	95.07 (270)	3.87 (11)	0 (0)	0 (0)	1.06 (3)
	F	270	93.33 (252)	4.81 (13)	0 (0)	1.85 (5)	0 (0)
	SA	40	95 (38)	2.50 (1)	0 (0)	0 (0)	2.50 (1)
	J	91	96.7 (88)	0 (0)	1.1 (1)	2.2 (2)	0 (0)
	Total	**685**	**94.60 (648)**	**3.65 (25)**	**0.15 (1)**	**1.02 (7)**	**0.58 (4)**
Teira dugesii jogeri	M	70	98.57 (69)	1.43 (1)	0 (0)	0 (0)	0 (0)
	F	27	100 (27)	0 (0)	0 (0)	0 (0)	0 (0)
	SA	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	J	3	100 (3)	0 (0)	0 (0)	0 (0)	0 (0)
	Total	**100**	**99 (99)**	**1 (1)**	**0 (0)**	**0 (0)**	**0 (0)**
Teira dugesii mauli	M	51	90.2 (46)	9.8 (5)	0 (0)	0 (0)	0 (0)
	F	49	93.88 (46)	6.12 (3)	0 (0)	0 (0)	0 (0)
	SA	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	J	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Total	**100**	**92 (92)**	**8 (8)**	**0 (0)**	**0 (0)**	**0 (0)**
Teira dugesii selvagensis (Selvagem Grande)	M	47	72.34 (34)	4.26 (2)	8.51 (4)	10.64 (5)	4.25 (2)
	F	25	76 (19)	0 (0)	8 (2)	16 (4)	0 (0)
	SA	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	J	1	0 (0)	0 (0)	100 (1)	0 (0)	0 (0)
	Total	**73**	**72.60 (53)**	**2.74 (2)**	**9.59 (7)**	**12.33 (9)**	**2.74 (2)**
Teira dugesii selvagensis (Selvagem Pequena)	M	14	14.29 (2)	0 (0)	14.29 (2)	71.43 (10)	0 (0)
	F	15	26.67 (4)	0 (0)	13.33 (2)	60 (9)	0 (0)
	SA	6	16.67 (1)	0 (0)	16.67 (1)	66.67 (4)	0 (0)
	J	8	25 (2)	0 (0)	25 (2)	50 (4)	0 (0)
	Total	**43**	**20.93 (9)**	**0 (0)**	**16.28 (7)**	**62.79 (27)**	**0 (0)**

Madeira Island samples, although the small number of individuals with this kind of pattern (5 females, 2 juveniles) prevents us from drawing further conclusions.

In all subspecies, occipital scaling patterns are distributed relatively uniformly between the sexes, with no significant variation. Type I appears more frequent in both sexes, while types II, III, and IV are less common and similarly distributed in the analysis of head scaling patterns. We observed that Type I is predominantly present in both males and females of *Teira dugesii dugesii,* with a *p*-value of 0.0640 in Fisher's Exact Test (Table 2). Although this value is close to the traditional threshold for statistical significance (0.05), it suggests a slight association between scaling patterns and sex. This trend is not sufficient to be considered statistically significant. The possible trend refers to a slight association between the head scaling patterns and sex in *Teira dugesii dugesii.* Figure 3 provides an overview of the distribution of head scaling patterns in both male and female individuals across the examined subspecies. The findings indicate that scaling Type I is the most prevalent in both sexes, accounting for the majority of the sample. This result suggests a strong consistency in head scaling patterns for this type, regardless of gender. In contrast, types II, III, and IV are less frequent overall. Notably, these types show a similar distribution among males and females, which indicates no significant bias or disparity between the sexes in these specific patterns. The data highlight a general uniformity in head scaling among the analyzed groups, with Type I the clear dominant pattern for both males and females.

In the multinomial logistic regression, Type I served as the baseline reference type, allowing for comparisons with types II, III, and IV. The overall model demonstrated statistical significance ($\chi^2 = 252.867$, $p < 0.001$), suggesting that certain subspecies exhibit significantly different frequency distributions of occipital scale patterns. Table 3 shows that no significant differences in the odds of Type II were observed for any subspecies compared to the baseline. For Type III, significant differences were found for Selvagem Grande and Selvagem Pequena, with both showing markedly higher odds of exhibiting reduced occipital scales compared to the baseline. For Type IV, both Selvagem Grande and Selvagem Pequena displayed significantly increased odds of having an absent occipital scale compared to the baseline.

During the study, some individuals with occipital deformities were found in five *Teira dugesii,* three *T. dugesii dugesii* from Madeira Island and two *T. dugesii selvagensis* from Selvagem Grande. Since four were males (one was a juvenile), it is possible that this is due to inter-male combat, although again numbers are too low to draw firm conclusions. Future studies should attempt to understand the real impact of fighting behavior on head scales and deformations due to developmental or genetic problems.

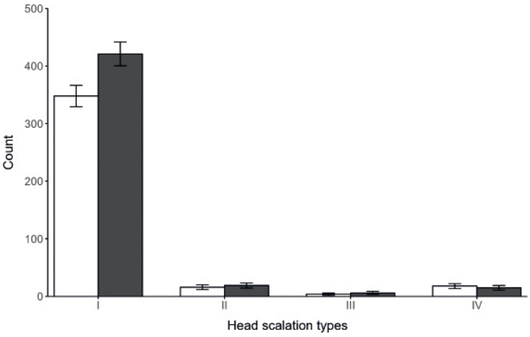

Figure 3. Distribution of head scaling patterns in the analyzed subspecies shows that Type I is dominant in both sexes, with types II, III, and IV being less common and similarly distributed. Error bars indicate confidence intervals, suggesting no clear sexual dimorphism in the head scale pattern (white bars; females; black bars: males).

Table 2. Summary of Fisher's Exact Test results assessing the association between head scalation types and sex categories across subspecies; *p*-values indicate whether significant differences exist within each subspecies.

Subspecies	*p*-value
Teira dugesii dugesii	0.0640
Teira dugesii jogeri	1.0000
Teira dugesii mauli	0.7155
Teira dugesii selvagensis (Selvagem Grande)	0.8762
Teira dugesii selvagensis (Selvagem Pequena)	0.8571

The original description by Milne-Edwards (1829) mentioned that *Teira dugesii* lacked occipital scales. According to Duméril and Bibron (1839) the description was based on 12 individuals collected in Madeira by Pierre Antoine Delalande. We believe that these specimens may have been captured in the 1810s during Delalande's visit to Portugal, on his way to an expedition to Brazil, possibly collected around the same time as the *Tarentola boettgeri bischoffi* Joger, 1984 (MNHN-

Table 3. Results of the multinomial logistic regression analysis examining the association between head scale types and subspecies. The reference type for the response variable was 'occipital scale present'. Coefficients (B), odds ratios (OR), and 95% confidence intervals (CI) are presented for each pattern type and subspecies. Significance values (*p*) are also shown.

Subspecies	Odds ratios and coefficients	Type II Occipital scale fragmented	Type III Occipital scale reduced	Type IV Occipital scale absent
Teira dugesii jogeri	B	-1.522	-5.540	-11.590
	OR	0.218	0.003	< 0.0001
	95%CL	0.029–1.618	< 0.0001–2.338 × 10^{32}	< 0.0001–2.838 × 10^{265}
	p	0.136	0.892	0.971
Teira dugesii mauli	B	0.640	-5.363	-11.434
	OR	1.878	0.005	< 0.0001
	95%Cl	0.836–4.222	< 0.0001–4.906 × 10^{30}	< 0.0001–3.519 × 10^{254}
	p	0.127	0.890	0.970
Teira dugesii selvagensis (Selvagem Grande)	B	-0.204	4.450	2.755
	OR	0.815	85.655	15.717
	95%CL	0.190–3.505	10.336–709.829	5.630–43.879
	p	0.784	< 0.001	< 0.001
Teira dugesii selvagensis (Selvagem Pequena)	B		6.223	5.626
	OR		504.426	277.686
	95%CL		56.066–4538.293	27.769–961.966
	p		< 0.001	< 0.001

RA-0.2276, MNHN-RA-2012.463) in 1816 (Gunn and Cood 1981).

Among the specimens of *Teira dugesii,* 10 individuals (MNHN-RA-0.2740, MNHN-RA-0.2740A (Figure 4), MNHN-RA-0.2740B, MNHN-RA-0.2740C, MNHN-RA-0.2742, MNHN-RA-0.2742A, MNHN-RA-0.2742B, MNHN-RA-0.7021, MNHN-RA-0.7021A, MNHN-RA-0.7021B) (Figure 5) were observed using photographs kindly provided by Dr. Nicolas Vidal, curator of Herpetology at the Muséum National d'Histoire Naturelle de Paris. Of these, only one individual lacked an occipital scale, MNHN-RA-0.2742A. This fact raises many doubts about how Milne-Edwards used these specimens to describe *Teira dugesii.* Duméril and Bibron (1839) expressed surprise at Milne-Edwards' description, noting that all the specimens they examined had an occipital plate. If Milne-Edwards has a set of individuals without occipital scales, it seems highly improbable that the specimens were collected on Madeira Island, given the reduced number of individuals that we found with Type IV characteristics. There is a possibility that these specimens originated from

Figure 4. The only individual without occipital scales (MNHN-RA-0.2742A) in Pierre Antoine Delalande's collection. Photo: Nicolas Vidal.

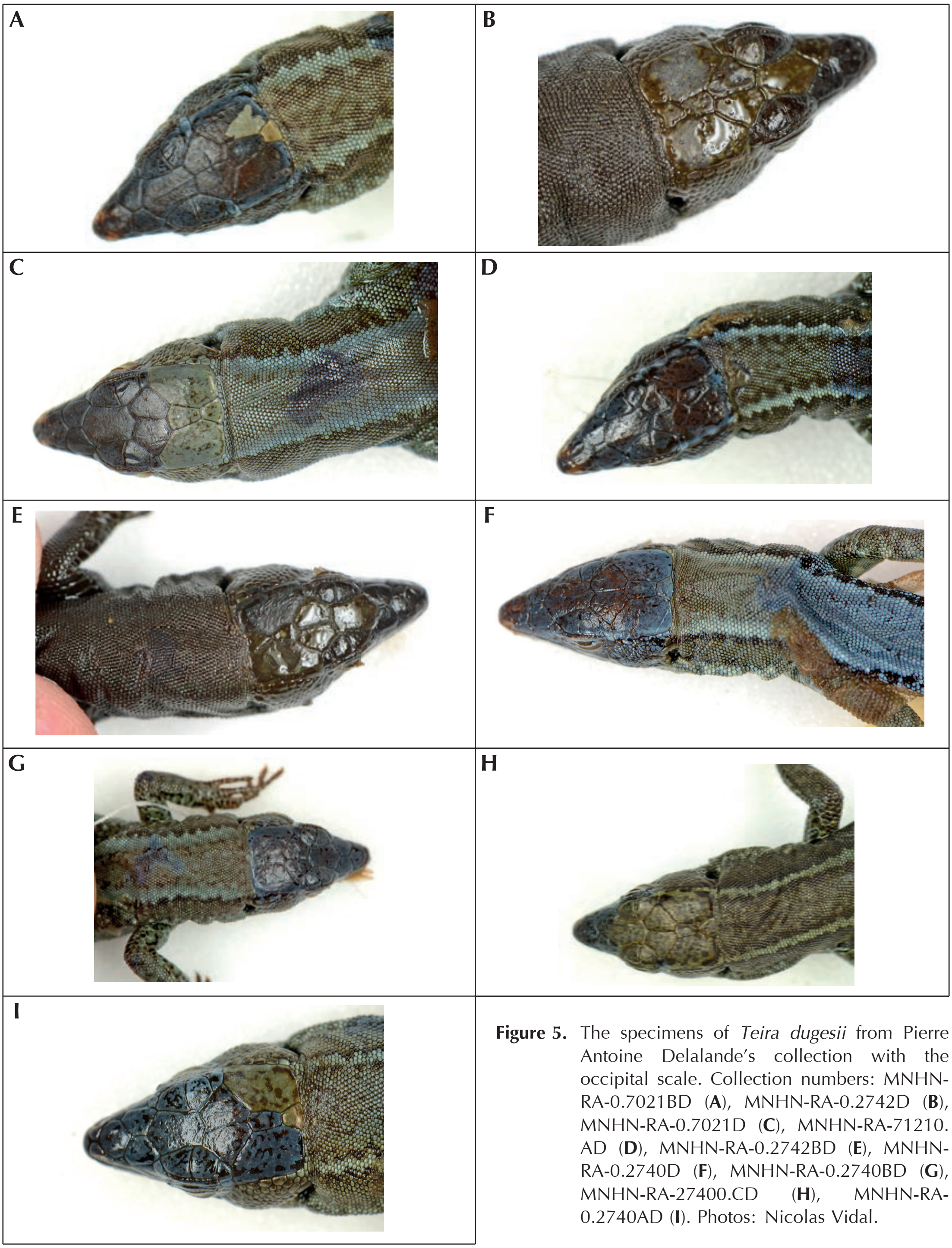

Figure 5. The specimens of *Teira dugesii* from Pierre Antoine Delalande's collection with the occipital scale. Collection numbers: MNHN-RA-0.7021BD (**A**), MNHN-RA-0.2742D (**B**), MNHN-RA-0.7021D (**C**), MNHN-RA-71210.AD (**D**), MNHN-RA-0.2742BD (**E**), MNHN-RA-0.2740D (**F**), MNHN-RA-0.2740BD (**G**), MNHN-RA-27400.CD (**H**), MNHN-RA-0.2740AD (**I**). Photos: Nicolas Vidal.

the Selvagens Islands, as the tags on the paralectotypes of *Tarentola boettgeri bischoffi,* collected by Pierre Antoine Delalande, indicate they were gathered in "Madère." It is conceivable that the reference to Madeira in this context also encompasses the Selvagens Islands. No historical records indicate that these reptiles were present on the island of Madeira, and they are known only from the Selvagens Islands. Similarly, the tags for the specimens used to describe the species *Teira dugesii* were exclusively labelled with "Madère."

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SHORT COMMUNICATION

Teira dugesii (Squamata: Lacertidae) visiting flowers: new observations and a review

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Keywords: Macaronesia, Madeiran Wall Lizard, Oceanic islands, Plant-lizard interactions.

Palavras-chave: Ilhas oceânicas, Interações planta-lagarto, Lagartixa-da-Madeira, Macaronésia.

Interactions between plants and lizards remain understudied, though it is widely acknowledged that these reptiles interact with flowering plants across various parts of the world (Correcher *et al.* 2023). Such interactions are particularly well-documented on islands, where lizard populations often reach high densities, and resources like arthropods are scarce (Buckley and Jetz 2007). This scarcity encourages lizards to broaden their diet to include alternative resources, such as flowers and fruits, allowing them to take on new ecological roles like pollination and seed dispersal (Pérez-Mellado 1989, Traveset and Sáez 1997, Pérez-Mellado and Casas 1997, Olesen and Valido 2003). This phenomenon, known as niche expansion or "ecological release" (Cox and Ricklefs 1977), is especially notable in lacertid species that were originally insectivorous but, upon colonizing islands, adapted to use a wider range of resources (Van Damme 1997).

By consuming nectar and fruit, lizards function as effective pollinators and dispersers if they visit the flowers and disperse the pollen and seeds to suitable locations (Traveset and Sáez 1997, Hansen and Müller 2009). In turn, plants provide essential resources for lizards such as water and nectar, which contains various nutrients, and insect prey on the flowers (Olesen and Valido 2003).

The Madeiran Wall Lizard, *Teira dugesii* (Milne-Edwards, 1829), is endemic to the Madeira archipelago and the Selvagens Islands. It has an average body size of 64 mm (Cook 1979). In recent years, multiple records have documented its visits to flowers, highlighting its potential ecological role in plant interactions across diverse habitats. The first record was by Elvers (1977), followed by subsequent studies reporting visits to various flowers, including *Musschia aurea* (L.) Dumort. (Elvers 1978, Olesen and Valido 2003, Olesen *et al.* 2002, 2012) and *Musschia isambertoi* M. Seq., R. Jardim, Magda Silva & L. Carvalho (Sequeira *et al.* 2007). The lizards have also been observed interacting with plants in other archipelagos, such as the Azores—*Azorina vidalii* (H. C.

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Wats.) Feer (Ferreira *et al.* 2016)—and the Selvagens—*Mesembryanthemum crystallinum* L., *Schizogyne sericea* (L.f.) DC., *Lotus glaucus* subsp. *selvagensis* (R. P. Murray) Sandral & D. D. Sokoloff (Ferreira *et al.* 2016). More recent studies have confirmed *Teira dugesii* as a pollinator of *Echium candicans* L.f. (Costa 2019, Esposito *et al.* 2021), further emphasizing its role in plant reproduction within its habitats.

In this study, we expand the existing data on interactions of *Teira dugesii* with flowers and compile all previously recorded observations of its floral visits. Observations were conducted over 75 days, totalling 101 hours. Most of the time, only one observer was present, and on rare occasions, two observers were involved. In such cases, one observer recorded data while the other observed and took photographs. Observations took place during three main periods: July–August 2023, March–May 2024, and July–August 2024, between 09:40 h and 17:20 h. No observations were conducted on rainy days. Additionally, some observations were obtained between February and early April 2025 during other field trips. We conducted the study on Madeira Island in various locations. We observed interactions at Rua Nova da Igreja (32°38'55" N, 16°53'04" W), Prazeres (32°45'15" N, 17°12'09" W), Miradouro de D. Sebastião (32°40'12" N, 17°03'46" W), Porto Novo (32°39'44" N, 16°48' 29" W), Jardim Botânico (32°39'46" N, 16°53'42" W), Rua Dr Fernando José Martins D'Almeida Couto (32°39'44" N, 16°55'51" W), Caminho da Ladeira (32°40'02" N, 16°56'19" W), Parque de Santa Catarina (32°38'43" N 16°54'51" W), and Lido (32°38'11" N, 16°56'04" W) (Figure 1). Although we conducted observations at several other locations, we did not record any lizard-flower interactions. These locations included Monte Palace Garden, Funchal Municipal Garden, Palheiro Gardens, São Martinho Garden, Encumeada Levada, Ponta de São Lourenço Trail, Madalenas Garden, Rua do Lazareto, Ajuda Garden, Amparo Garden, Larano Levada, and Santana Theme Park. This result likely occurred because *Teira dugesii* has a highly varied diet (Sadek 1981) and may choose other food sources. In areas with dense forests, the low number or absence of lizards reduced the likelihood of these interactions (pers. obs.). The interactions we observed took place in areas where lizards had abundant sunlight exposure and access to herbaceous and shrubby vegetation.

We collected data according to the different stages of flowering. To avoid influencing the lizards' behavior, we maintained a distance of 4 m from the plants, as closer approaches typically caused the lizards to flee. This precaution was essential to allow natural interaction behaviors to be observed without human interference. Each observation lasted approximately 30–45 minutes and was repeated on different days to confirm that this behavior was common. Not all locations were revisited. We used a Nikon P1000 camera to capture photographs and recordings.

During the interactions, we recorded various behaviors, including nectar feeding. The lizards' approach varied depending on the plant species. On plants with inflorescences, the lizards tended to climb directly onto them, while on species with single flowers, such as *Hibiscus rosa-sinensis* L., they often secured their tails around the stamen to prevent falling. We observed licking movements, as well as alert postures where the head and anterior body were elevated, the tail was fully extended and resting, and the eyes remained open. Additionally, incidental passages occurred when the lizards used the flowers as stepping points to reach another stem or plant.

We conducted a literature review to identify studies and records on the floral visitation of *Teira dugesii,* with a particular focus on Madeira Island. Using the Web of Science and Google Scholar databases, we searched with the following combination of keywords and Boolean operators: ('lizard' OR 'reptile') AND ('flower visit in Madeira Island' OR 'pollination' OR 'nectar' OR 'nectarivory' OR 'flower' AND 'diet').

We included peer-reviewed articles and studies that specifically examined interactions

Figure 1. Locations of observations of the interactions between *Teira dugesii* and flowers on Madeira Island in this study.

between *Teira dugesii* and plants or analyzed its diet involving nectar consumption or flower visits. To ensure comprehensive coverage, we applied no time restrictions, incorporating both historical and contemporary studies.

Through our direct observations and analysis, we documented interactions between lizards and the flowers of 34 plant species, 30 of which were newly recorded on Madeira Island (Table 1). Approximately 88.2% of these interactions are novel. Our study accounts for 69.7% of the recorded plant-lizard interactions. The most frequently visited species in this study were *Foeniculum vulgare* Mill., *Crithmum maritimum* L., and *Hibiscus rosa-sinensis,* followed by *Rhododendron* spp. and *Opuntia* spp. (Table 2).

The Madeiran wall lizards displayed varied interactions with different flowers, but legitimate visits were by far the most common across all plant species, accounting for 97% of the observations, while illegitimate visits represented only 3%. A visit was considered legitimate when the lizard directly approached the flower to engage in nectar-feeding behavior, such as licking nectar, or in cases like *Opuntia* spp., where lizards also used the flowers for resting and thermoregulation. In contrast, visits were classified as illegitimate when the lizard had no apparent intention of interacting with the flower but made incidental contact with it, such as stepping on the petals while moving through the vegetation. None of the documented interactions involved behavior that damaged the plants' reproductive organs, such as the florivory observed in *Hibiscus rosa-sinensis* (Câmara and Jesus *In press*).

This study documents new flower–lizard interactions in 30 plant species, suggesting that the Madeiran wall lizard (*Teira dugesii*) may be the lacertid species with the highest number of recorded floral interactions. Comparable records are scarce, with few studies documenting more than 20–40 interactions per species, and only Romero-Egea *et al.* (2023) reporting similarly high numbers of records.

Among the 30 species, three are endemic: *Aeonium glutinosum* (Aiton) Webb & Berthel. (Madeira archipelago and Selvagens

Table 1. Data on visits of *Teira dugesii* to flowers. Legend: End, endemic; I, introduced; N, native, Orn, ornamental, M, Macaronesia; NL, no location.

Plant Species	Common name	Origin	Type of interaction	Location	Known on Madeira and Selvagens arquipelagos	Source
Agavaceae						
Agave attenuate	Foxtail Agave	I		Garajau, Santa Cruz	Yes	Elvers 1977
Aizoaceae						
Carpobrotus edulis	Hottentot Fig	I	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Mesembryanthe mum crystallinum	Crystalline Iceplant	I		Selvagem Grande	Yes	Ferreira *et al.* 2016
Apiaceae						
Chrithmum maritimum (Figure 3N)	Sea Fennel	N	Legitimate visit	Lido, Funchal	No	**This study**
Foeniculum vulgare (Figure 3F)	Fennel	N	Legitimate visit	Miradouro de S. Sebastião, Ribeira Brava	Yes	Sziemer 2010; **This study**
Apocynaceae						
Allamanda cathartica (Figure 3P)	Golden Trumpet Vine	Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Asteraceae						
Cynara cardunculus	Artichoke Thistle Cardoon	N		NL	Yes	Beyhl 1990
Schizogyne sericea	Salado Blanco	M		Selvagem Grande	Yes	Ferreira *et al.* 2016
Begoniaceae						
Begonia sp. (Figure 3A)		Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Boraginaceae						
Echium candicans	Pride of Madeira	End	Legitimate visit	Pico do Arieiro	Yes	Ricardo Costa 2019, Esposito *et al.* 2021
Echium nervosum (Figure 3L)	Coastal Pride of Madeira	End	Legitimate visit	Garajau, Santa Cruz and Caminho Real do Paul do Mar	Yes	Elvers 1977; **This study**
Cactaceae						
Opuntia spp. (Figure 3J)	Cactus Pear	I	Legitimate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**

Table 1. *Continued.*

Plant Species	Common name	Origin	Type of interaction	Location	Known on Madeira and Selvagens arquipelagos	Source
Campanulaceae						
Musschia aurea	Madeira Giant Bellflower	End		Garajau, Santa Cruz and São Gonçalo, Funchal	Yes	Elvers 1978, Olesen and Valido 2003, Olesen *et al.* 2012
Musschia isambertoi		End		Desertas islands	Yes	Sequeira *et al.* 2007
Tracheoulium caeruleum	Blue Throatwort	I		NL	Yes	Beyhl 1990
Crassulaceae						
Aeonium glutinosum	Viscid Houseleek	End	Legitimate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**
Kalanchoe spp.		Orn	Legitmate visit	Caminho da Ladeira	No	**This study**
Echeveria atropurpurea	Chapetona	Orn		Garajau, Santa Cruz	Yes	Elvers 1977
Ericaceae						
Rhododendron spp. (Figure 3O)	Macranthum Azalea	Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Euphorbiaceae						
Euphorbia milii	Crowns of Thorns	Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Euphorbia piscatoria (Figure 3Q)	Fish-Stunning Spurge	End	Legitimate visit	Porto Novo, Santa Cruz	No	**This study**
Euphorbia pulcherrina	Poinsettia	Orn		Funchal	Yes	Beyhl, 1990
Ricinus communis	African Wonder Tree	I	Legitmate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**
Fabaceae						
Bituminaria bituminosa	Arabian Pea	N	Legitimate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**
Lotus glaucus sub selvagensis		End		Selvagem Grande	Yes	Ferreira *et al.* 2016
Geraniaceae						
Pelargonium sp.		I		Funchal	Yes	Beyhl 1990

Table 1. *Continued.*

Plant Species	**Common name**	**Origin**	**Type of interaction**	**Location**	**Known on Madeira and Selvagens arquipelagos**	**Source**
Hydrangeaceae						
Hydrangea macrophylla (Figure 3D)	Big Leaf Hydrangea	I	Legitimate visit	São Vicente and Igreja de Nossa Senhora das Neves, Calheta	Yes	Petrovan 2023; **This study**
Iridiaceae						
Gladiolus spp.		Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Lamiaceae						
Globularia salicina	Globe Daisy Shrub	Mac	Legitimate visit	Ribeira do Natal, Caniçal	No	**This study**
Leonotis leonurus	Lion's Tail	Orn	Illegitimate visit	Lido, Funchal	No	**This study**
Salvia rosmarinus (Figure 3E)	Rosemary	Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Salvia splendens	Scarlet Sage	Orn		Garajau, Santa Cruz	Yes	Elvers 1977
Liliaceae						
Aloe arborescens	Krantz Aloe	I		Garajau, Santa Cruz	Yes	Elvers 1977
Malvaceae						
Hibiscus rosa-sinensis (Figure 3K)	Chinese Hibiscus	Orn	Legitimate visit	Rua Nova da Igreja, Funchal and R. Dr Fernando José Martins D'Almeida Couto	No	**This study**
Moraceae						
Ficus carica	Common Fig	I	Legitimate visit	Rua do Lazareto, Funchal	No	**This study**
Myrtaceae						
Leptospermum scoparium	Tea Tree	I	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
Melaleuca regulosa (Figure 3R)	Scarlet Bottlebrush	Orn	Legitimate visit	Parque de Santa Catarina	No	**This study**
Psidium cattleyanum	Strawberry Guava	I	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**

Table 1. *Continued.*

Plant Species	**Common name**	**Origin**	**Type of interaction**	**Location**	**Known on Madeira and Selvagens arquipelagos**	**Source**
OLEACEAE						
Ligustrum sinense (Figure 3B)	Chinese Privet	Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
ORCHIDACEAE						
Phalaenopsis spp. (Figure 3M)		Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
POLYGONACEAE						
Polygonum capitatum	Pink Clover	I	Illegitimate	Jardim Botânico, Funchal	No	**This study**
ROSACEAE						
Rosa spp. (Figure 3C)		Orn	Legitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
SAPINDACEAE						
Cardiospermum grandiflorum (Figure 3H)	Showy Balloonvine	I	Illegitimate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**
SOLANACEAE						
Capsicum annuum	Red pepper	I	Legitimate visit	Caminho da Ladeira	No	**This study**
Nicotiana glauca (Figure 3G)	Tree Tobacco	I	Legitimate visit	Funchal, Miradouro de S. Sebastião, Ribeira Brava	Yes	Beyhl 1990; **This study**
STRELITZIACEAE						
Strelitzia reginae (Figure 3I)	Bird of Paradise	Orn	Legitimate visit	Miradouro de S. Sebastião, Ribeira Brava	No	**This study**
URTICACEAE						
Parietaria judaica	Pellitory-of-the-Wall	N	Illegitimate visit	Rua Nova da Igreja, Funchal	No	**This study**
VERBENACEAE						
Lantana camara	Common Lantana	I	Illegitimate visit	Lido, Funchal	No	**This study**

Table 2. Flower visitation frequency by *Teira dugesii.*

Plant	Visits	Percentages
Foeniculum vulgare	101	46.54%
Chrithmum maritimum	17	7.83%
Hibiscus rosa-sinensis	16	7.37%
Rhododendron spp.	13	5.99%
Opuntia spp.	8	3.69%
Euphorbia milii	7	3.23%
Allamanda cathartica	4	1.84%
Echium nervosum	4	1.84%
Hydrangea macrophylla	4	1.84%
Ligustrum sinense	4	1.84%
Ficus carica	3	1.38%
Salvia rosmarinus	3	1.38%
Leptospermum scoparium	3	1.38%
Strelitzia reginae	3	1.38%
*Melaleuca regulos*a	3	1.38%
Begonia sp.	2	0.92%
Euphorbia piscatorial	2	0.92%
Globularia salicina	2	0.92%
Phalaenopsis spp.	2	0.92%
Rosa spp.	2	0.92%
Capsicum annum	2	0.92%
Carpobrotus edulis	1	0.46%
Ricinus communis	1	0.46%
Bituminaria bituminosa	1	0.46%
Gladiolus spp.	1	0.46%
Leonotis leonurus	1	0.46%
Aeonium glutinosum	1	0.46%
Psidium cattleyanum	1	0.46%
Polygonum capitatum	1	0.46%
Cardiospermum grandiflorum	1	0.46%
Nicotiana glauca	1	0.46%
Parietaria judaica	1	0.46%
Kalanchoe spp.	1	0.46%

Islands), *Globularia salicina* Lam. (Macaronesia), and *Euphorbia piscatoria* Aiton (Madeira Archipelago and Selvagens Islands). Additionally, three are native: *Crithmum maritimum*, *Bituminaria bituminosa* (L.) C. H. Stirt., and *Parietaria judaica* L. The remaining species are introduced or ornamental.

The plants most visited by *Teira dugesii* have flowers that appear close together or grouped in inflorescences**.** Therefore, the family with the most visits was the Apiaceae (*Foeniculum vulgare, Chrithmum maritimum*). Romero-Egea *et al.* (2023) observed that the most visited plants were *Daucus* sp. and *Lobularia* sp. with these plants having inflorescences and close flowers. *Foeniculum vulgare* was the plant species with the highest number of recorded lizard visits. Notably, it was also where juvenile lizards were most frequently observed accessing the flowers. We cannot conclusively state that juveniles are the primary visitors because we did not estimate the relative densities of different age classes. Nonetheless, this observation aligns with findings by Sziemer (2010), who reported a similar pattern. We observed that approximately 95% of the visitors were juveniles and subadults. We observed intriguing behaviors, including repeated instances of aggressive interactions on three different days as individuals competed for access to inflorescences (Figures 2 and 3). Although juveniles and subadults were the most frequent floral visitors, we also recorded adult males on flowers, albeit less frequently. Adult males were observed visiting *Opuntia* sp. (twice), *Foeniculum vulgare* (three times), and *Hibiscus rosa-sinensis* (three times).

Of these 30 species, we recorded interactions with three endemic plants—*Euphorbia piscatoria, Globularia salicina,* and *Aeonium glutinosum*—highlighting the need for more detailed studies on the potential benefits of these endemic lizards for the survival of native flora in Madeira Island. We recorded several introduced plant species being visited by lizards, which may play a role in their dispersal. Among the 34 plant species observed, 26 (76.5%) were ornamental and introduced, highlighting the potential influence of lizards in the spread of non-native flora in the ecosystem.

Figure 2. Aggressive behavior between two *Teira dugesii* (Photo: JC).

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Figure 3. *Teira dugesii* visiting the flower of *Begonia* sp. (**A**), foraging in the flowers of *Ligustrum sinense* (**B**), visiting the flower of *Rosa* spp. (**C**), visiting the inflorescence of *Hydrangea macrophylla* (**D**), licking the nectar of *Salvia rosmarinus* (**E**), licking the nectar of *Foeniculum vulgare* (**F**), licking the nectar of *Nicotiana glauca* (**G**), visiting the flower of *Cardiospermum grandiflorum* (**H**), visiting the flower of *Strelitzia reginae* (**I**), visiting the flower of *Opuntia* spp. (**J**), licking the nectar of *Hibiscus rosa-sinensis* (**K**), visiting *Echium nervosum* (**L**), visiting the flower of *Phalaenopsis* sp. (**M**), licking the nectar of *Chrithmum maritimum* (**N**), licking the nectar of *Rhododendron* spp. (**O**), and visiting the flower of *Allamanda cathartica* (**P**), licking the nectar of *Euphorbia piscatoria* (**Q**), licking the nectar of *Melaleuca regulosa* (**R**). Photos: JC.

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SHORT COMMUNICATION

The first evidence of viviparity in the lizard *Liolaemus reichei* (Squamata: Liolaemidae)

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Keywords: Chile, Desert, Dystocia, Reproductive biology.

Palabras claves: Biología reproductiva, Chile, Desierto, Distocia.

Palavras-chave: Biologia reprodutiva, Chile, Deserto, Distocia.

Studying the reproductive biology of an organism is fundamental to understanding its life history and adaptations to different environments (Packard *et al.* 1977). In the case of reptiles, given their condition as ectothermic vertebrates, reproduction and embryo gestation of species that inhabit temperature-challenged environments face diverse physiological defiances (Shine 2004). The evolution of viviparity (live breeding) from oviparity (egg-laying) may be an adaptive response to extreme climates, allowing females to regulate the embryo temperature and thus keep it under stable conditions in contrast to the comparatively higher thermal variation to which eggs are exposed in the environment (Shine 2005, Fernández *et al.* 2017).

Lizards in the genus *Liolaemus* Wiegmann, 1834 inhabit the southern cone of South America and are highly diverse, with almost 300 species (Uetz *et al.* 2025). They occur in a variety of habitats, from hot areas on the Atlantic coast of Brazil, and hot deserts in Peru and Chile to the cold regions in Argentine Patagonia and the highlands (~ 5000 m a.s.l.) of the Central Andes of Peru and Bolivia (Abdala and Quinteros 2014, Abdala *et al.* 2020, Cerdeña *et al.* 2021). Data for this genus support the hypothesis that viviparity evolves in cold climates because this reproductive mode is primarily observed in high altitudes and latitudes (Pincheira-Donoso *et al.* 2013, Esquerré *et al.* 2019). However, viviparity has also been observed in species that inhabit hot deserts (Abdala *et al.* 2021b) with extreme climatic conditions, such as high daily temperature oscillations and low precipitation (Weischet 1975, Valdivia-Silva *et al.* 2012). Under these conditions, viviparity would not only protect embryos against significant thermal variation but also against water stress (Shine and Thompson 2006). This situation may be the case for the species of the *Liolaemus reichei* clade (*sensu* Abdala *et al.* 2020, Valladares *et al.* 2021) that inhabit the desert zones of southern Peru and northern Chile. Viviparity has been reported

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for seven of the 12 species in this clade: *L. anqapuka* Huamani-Valderrama, Quiróz, Gutiérrez, Aguilar-Kirigin, Chaparro, and Abdala, 2020 (Huamaní-Valderrama and Quiróz 2021), *L. audituvelatus* Núñez and Yáñez, 1983 (Valladares-Faúndez and Briones 2012), *L. balagueri* Villegas Paredes, Huamani-Valderrama, Luque-Fernández, Gutiérrez, Quiróz, and Abdala, 2020 (Huamani-Valderrama and Gutiérrez 2021), *L. chiribaya* Aguilar-Puntriano, Ramírez, Castillo, Mendoza, Vargas, and Sites, 2019 (Quiroz and Huamani-Valderrama, 2021), *L. insolitus* Cei and Péfaur, 1982 (Abdala *et al.* 2021a), *L. nazca* Aguilar-Puntriano, Ramírez, Castillo, Mendoza, Vargas, and Sites, 2019 (Aguilar-Puntriano *et al.* 2019), and *L. torresi* Núñez, Navarro, Garín, Pincheira-Donoso, and Meriggio, 2003 (Núñez *et al.* 2003). The prevalence of viviparity in this clade suggests that this reproductive mode may be an ancestral character and that the other species of the clade may also be viviparous. Of the five species for which reproductive mode is unknown, one is *Liolaemus reichei* Werner, 1907 (Valladares-Faúndez and Abdala 2021, but see Donoso-Barros 1966), considered a junior synonym of *L. stolzmanni* Steindachner, 1891 and revalidated a few years ago as a full species (Langstroth 2011, Valladares-Faúndez *et al.* 2018, Troncoso-Palacios and Escobar 2020). This species, listed as endangered by the IUCN, is small, with a maximum snout–vent length (SVL) of 50.82 mm, has non-evident sexual dichromatism, and precloacal pores are present only in males (Valladares and Abdala 2021). It inhabits absolute desert, occupying terrestrial biotopes with sandy and stony substrates (Valladares-Faúndez and Abdala 2021). In the present study, we report field observations that reveal viviparity in this species.

As part of monthly monitoring to study *L. reichei* in a desert area (Figure 1) located 70 km southeast of Iquique (20°49'16" S, 69°57'09" W) in the Tarapacá Region of northern Chile, on 16 January 2024 at 13:20 h we captured an adult pregnant female under a rock. Its reproductive state was determined based on the bulging of her abdomen (Figure 2). Her SVL was 48.91 mm, and her tail length (TL) was 32.93 mm, both measured with a Proster® electronic caliper (± 0.01 mm). The female's weight was 4.3 g, recorded with a MH Pocket digital scale® (± 0.1 g). Following the monitoring protocol, the female was georeferenced using the phone App Locus map® version 4.28.3, marked with non-toxic paint, and photographed with a Canon EOS 90D® camera before it was released at the site of capture.

Two months later, on 11 March 2024 at 14:37 h, this female was recaptured under a rock, approximately 100 m from the previous site. It was recognized by a characteristic malformation in the tail revealed when photographs were inspected. Before starting the routine measurements, we noticed a neonate head protruding from the female's cloaca. The lizard was placed back under the rock to allow expulsion of the neonate. After one hour, we returned to find no change in the neonate position. Close examination confirmed that this individual was dead (Figure 3). The female was left under the rock, allowing expulsion of the stillborn. The next day, 12 March, at 10:50, the female was found under a different rock located 30 m from where it was found the day before. Because the stillborn was not yet expelled, we assisted the female in its expulsion, thus preventing her death. The posterior part of the female's body was placed in a container with warm water (~35°C) to promote dilatation of the cloacal region. Together with a controlled massage of the abdominal region and the application of petroleum jelly with a cotton swab in the cloacal area, the stillborn was finally expelled, a procedure that lasted approximately two minutes. The female and the stillborn were weighed separately; these values were 2.5 and 0.5 g, respectively. The SVL of the stillborn was 31.03 mm, and its TL was 26.68 mm. The stillborn lizard was collected for future research. Further analysis of the female reproductive condition was not performed (e.g., palpations,

Figure 1. The desert environment in the Tarapacá region inhabited by *Liolaemus reichei.*

Figure 2. Pregnant female of *Liolaemus reichei.*

Figure 3. Cloacal area of the pregnant female of *Liolaemus reichei* with an emerging neonate (stillborn).

ultrasounds) to minimize the intervention. After that, the female was released and ran actively to hide under the same rock where she had been found earlier. Two months later, on 12 May at 13:55 h, this female was recaptured under a rock located approximately 130 m from the last capture. It actively attempted to bite and escape while handled. At this time, her weight was 2.6 g. Finally, on 7 February 2025 at 15:15 h, the female was recaptured approximately 60 m from the last capture point and weighed 3.3 g.

These observations confirmed viviparity in *L. reichei,* which is consistent with the occurrence of this reproductive mode in other species of the *L. reichei* clade. However, our observations contradict the proposition that the species is oviparous (Donoso-Barros 1966). This discrepancy may be rooted, as Núñez *et al.* (2003) indicated, because Donoso-Barros (1966) observed oviductal eggs at an early stage of pregnancy. Our observations suggest that births in *L. reichei* occur in Austral summer, partially coincident with the parturition of *L. audituvelatus* recorded in late spring (Valladares and Briones 2012). Nevertheless, data from the ongoing monitoring revealed the presence of small individuals (< 35 mm) between October and May, suggesting that the species may have an extended breeding season. Finally, it should be noted that the neonate emerged head-first, as has been observed in other species of *Liolaemus* (Halloy and Halloy 1997, Cabrera and Monguillot 2007, Minoli *et al.* 2010, Valladares-Faúndez and Briones 2012).

Valladares-Faúndez and Briones (2012) indicated that in *L. audituvelatus,* parturition lasted 63 min, with birth intervals between the three offspring of 14 and 49 min. In other viviparous species of *Liolaemus,* the reported duration of parturition ranged between 38 and 100 min, with variable birth intervals between seven and 60 min, for litter sizes of two to seven individuals (Halloy and Halloy 1997, Ibargüengoytía *et al.* 2002, Cabrera and Monguillot 2007, Kozykariski *et al.* 2008, Minoli *et al.* 2010, Fernández *et al*. 2015). In this context, our decision to wait 60 min for the end of the neonate expulsion was appropriate. On the other hand, considering that the pregnant female weighed 4.3 g in January 2024, but after the (induced) parturition weighed 2.5 g, and that the stillborn mass was 0.5 g, we postulate that the female had previously given birth to one or two neonates. This litter size is in line with litter sizes reported for other species of the *L. reichei* clade: *L. audituvelatus* (*N* = 3; Valladares-Faúndez and Briones 2012), *L. insolitus* (*N* = 3; Abdala *et al.* 2021a), *L. nazca* (*N* = 2; Aguilar-Puntriano *et al*. 2019) and *L. torresi* (*N* = 2; Núñez *et al.* 2003). Since no other newborns were observed in the vicinity, presumably, the birth of the other neonates would have occurred several hours or days before our observation. In *L. audituvelatus* and *L. kingii* Bell, 1843 newborns initially remain after birth in the vicinity of the female (Ibargüengoytía *et al.* 2002, Valladares-Faúndez and Briones 2012).

Dystocia is an abnormal, slow, or difficult parturition, which is a reproductive disorder commonly reported in different species of reptiles in captivity. Egg retention is the most common cause in snakes, turtles, and lizards, such as iguanas and geckos (DeNardo *et al.* 2000, Lock 2000, Sykes 2010). Dystocia is classified as obstructive and non-obstructive, and its causes are multifactorial. Obstructive dystocias are associated with anatomical abnormalities (fetal or maternal) that constrain the passage of eggs or young through the oviduct, uterus, or cloaca but also can be associated with external factors related to the reproductive system that compress the passage of eggs or young through the birth canal (e.g., neoplasia, narrow pelvis, fecal bolus, renomegaly). Non-obstructive dystocias are the most common cases reported in captivity and are caused by inappropriate environmental conditions for parturition or egg laying, such as lack of nesting sites, inadequate environmental conditions (e.g., temperature, humidity, substrate, photoperiods), poor physical condition of females, inadequate diet, or early birth (DeNardo *et al.* 2000, Lock 2000, DeNardo 2006, Sykes 2010). The causes of most dystocias remain unresolved (DeNardo *et al.* 2000). In the case of *L. reichei,* it was not possible to determine the type of dystocia. However, because the stillborn had a similar size as the neonates of *L. torresi* reported by Núñez *et al.* (2003), a species of the *L. reichei* clade with a similar size as *L. reichei* (M. Weymann, pers. obs.), we ruled out the possibility that the neonate was premature. Other factors may have determined the neonate's death. Our observation may have resulted from non-obstructive dystocia due to extreme climatic conditions and low food availability where *L. reichei* lives (Donoso-Barros 1966). These conditions may have negatively affected the female at the time of parturition. Nevertheless, other observations indicate that the female was not moribund or lacking energy. It was able to move between shelters (~ 30 m) while still carrying the stillborn, was shedding during the parturition period (Figure 3), and after the assisted parturition, ran actively. In addition, during each handling, it struggled to escape. The female was recaptured two and 11 months later and had gained weight from 2.5 to 2.6 g to 3.3 g, respectively. Therefore, the factors involved in this dystocia are unclear.

Observations of dystocia in reptiles in the wild are rare (DeNardo *et al.* 2000). Our study of *L. reichei* not only revealed the reproductive mode of this species but also is the first case of dystocia reported for the species, as well as for the genus.

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SHORT COMMUNICATION

First record of the ixodid tick *Amblyomma nitidum* on *Laticauda laticaudata* (Serpentes: Elapidae) in Taiwan

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Palavras-chave: Ectoparasitas, Krait-do-mar-de-lábios-azuis, Laticaudinae, Serpentes marinhas.

The genus *Laticauda* Laurenti, 1768 is a small group of semi-aquatic elapid snakes native to the Indo-Malayan faunal region, although the ranges of some species extend to Australasia. This is the only genus in the Laticaudinae (Sea Kraits), one of the two extant subfamilies of sea snakes (the other is subfamily Hydrophiinae) (Rasmussen *et al.* 2011, 2014). Worldwide, there are eight extant species of sea kraits (Uetz *et al.* 2024), and they are typically found in tropical shallow coastal habitats, coral reefs, and mangroves (Voris and Voris 1983). Unlike "true" sea snakes (e.g., genus *Hydrophis* Latreille,1801), which copulate in the sea and are viviparous (Sanders *et al.* 2013), sea kraits need to come on land to mate and lay eggs (Klemmer 1966, Heatwole 1999). In Taiwan, three species of sea kraits have been reported: *L. colubrina* (Schneider, 1799) (Yellow-lipped Sea Krait), *L. laticaudata* (Linnaeus, 1758) (Blue-lipped Sea Krait), and *L. semifasciata* (Reinwardt, 1837) (Black-banded Sea Krait) (Tu *et al.* 1990, Liu *et al.* 2012) (Figure 1).

During a scuba diving exercise conducted on 10 June 2024 in Gongliao District, New Taipei City, Taiwan (25.114193° N, 121.920320° E; WGS 84), an adult specimen of *L. laticaudata* was observed foraging near the sea floor. Upon closer inspection, a tick was observed attached to an area of traumatic injury laterally on the body. The tick specimen was carefully removed by one of the divers and sent to the Herpetology Laboratory of National Taiwan Normal University (Taipei, Taiwan) for identification. The specimen was examined using a Leica S9D stereomicroscope and identified as a female *Amblyomma nitidum* Hirst and Hirst, 1910 based on the descriptions and keys in Voltzit and Keirans (2002). The tick voucher specimen will

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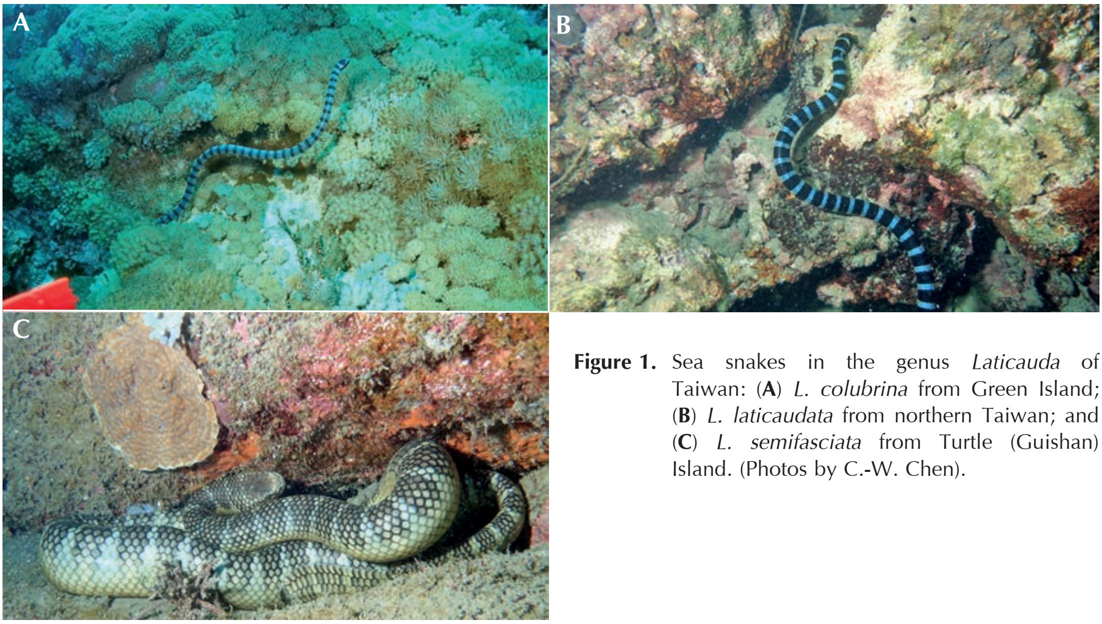

Figure 1. Sea snakes in the genus *Laticauda* of Taiwan: (**A**) *L. colubrina* from Green Island; (**B**) *L. laticaudata* from northern Taiwan; and (**C**) *L. semifasciata* from Turtle (Guishan) Island. (Photos by C.-W. Chen).

be deposited in the collections of the National Museum of Natural Science (Taichung, Taiwan). In addition, a female *A. nitidum* was photographed attached to the tail of an adult *L. colubrina* at a different site in Gongliao District, New Taipei City, Taiwan (25.116879° N, 121.916950° E; WGS 84) on 14 July 2020 (Figures 2–4).

Amblyomma nitidum is a rarely encountered species of inornate bont tick native to the Oriental and Australasian faunal realms. This species is widely distributed in the western Pacific, extending to the Andaman Islands in the Indian Ocean (Petney *et al.* 2019). Interestingly, it is one of the few Asiatic *Amblyomma* ticks that are exclusively associated with a single host group, elapid snakes belonging to the highly venomous, amphibious genus *Laticauda* (Kwak *et al.* 2020). Despite the wide geographic range of its hosts, published accounts of *A. nitidum* are relatively few, as this species is rarely encountered and collected. Country reports for *A. nitidum* include India (Andaman Islands) (Sharif 1928, De and Sanyal 1984), Japan (Yamaguti *et al.* 1971, Takano *et al.* 2014), New Caledonia (Rageau 1967, Bonnet 2012), Papua New Guinea (Wilson 1970), Singapore (Warburton 1933), and the Solomon Islands (Hirst and Hirst 1910). In Taiwan, *A. nitidum* was first reported by Kwak *et al.* (2020), who collected two adult male specimens on *L. colubrina* from Sanxiantai Island (Taitung County, southeast Taiwan). Our account constitutes the second published record of *A. nitidum* from Taiwan and is the first report of this tick from *L. laticaudata* in Taiwan. Further research should yield records of *A. nitidum* parasitizing *L. semifasciata* in Taiwan and should extend the range of this tick species to such archipelagic countries as Indonesia and the Philippines, where suitable habitats exist (e.g., rocky intertidal areas) for both sea snakes and ticks.

Although rarely studied, sea snakes are host to several endoparasitic and ectoparasitic taxa (e.g., Schmidt and Kuntz 1973, Hayashi and Masunaga 2001, Gillet *et al.* 2016), and even

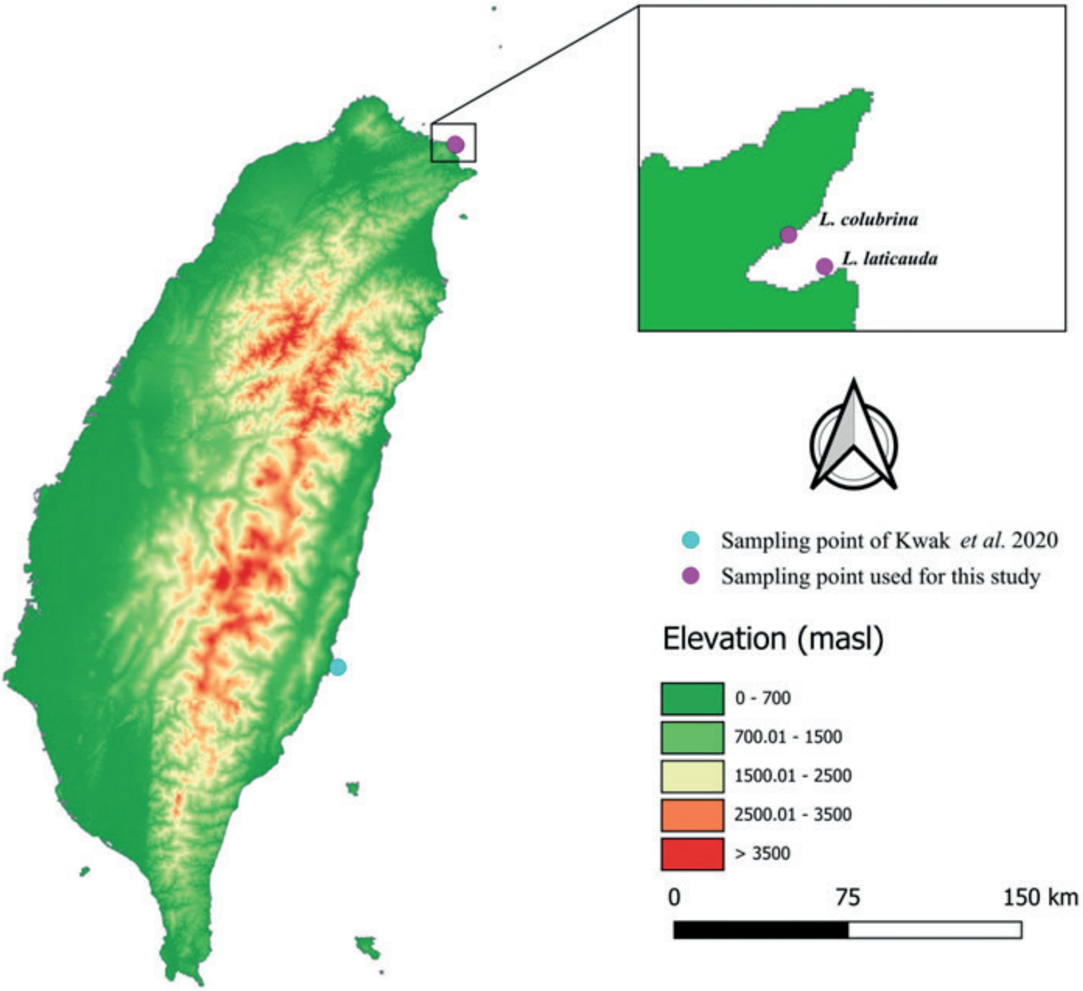

Figure 2. Updated locality records of *Amblyomma nitidum* reported from Taiwan.

marine epibionts (Key Jr. *et al.* 1995, Pfaller *et al.* 2012). These parasitic assemblages range from unicellular haemogregarines infecting erythrocytes (Qiu *et al.* 2021) to several species of helminths (Coil and Kuntz 1960, Vercammen-Grandjean and Heyneman 1964, Chinnadurai *et al.* 2008, Choe *et al.* 2020), acarines (Hirst and Hirst 1910, Nadchatram 2006, Takahashi *et al.* 2012), and cymothoid isopods (Saravanakumar *et al.* 2011). In addition, severe cases of barnacle epibiosis have been reported on sea snakes (Gillet *et al.* 2014, 2017). Aside from *A. nitidum,* which is the only tick known to parasitize sea kraits, a few species of trombiculid mites have evolved as parasites of the genus *Laticauda,* including *Ancoracarus hayashii* Takahashi, Misumi and Takahashi, 2012, *Iguanacarus alexfaini* Nadchatram, 1980, *Vatacarus ipoides* Southcott, 1957, and *Schoutedenichia masunagai* Takahashi, Misumi and Takahashi, 2012

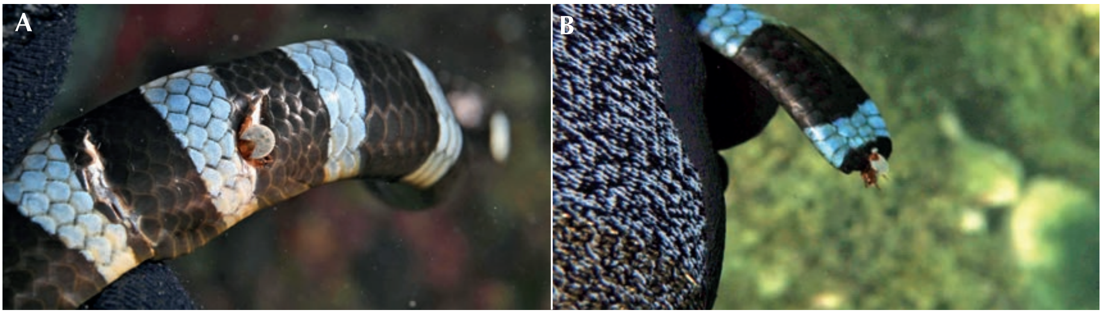

Figure 3. *Amblyomma nitidum* attached in situ on their hosts, *Laticauda laticaudata* (**A**) and *L. colubrina* (**B**).

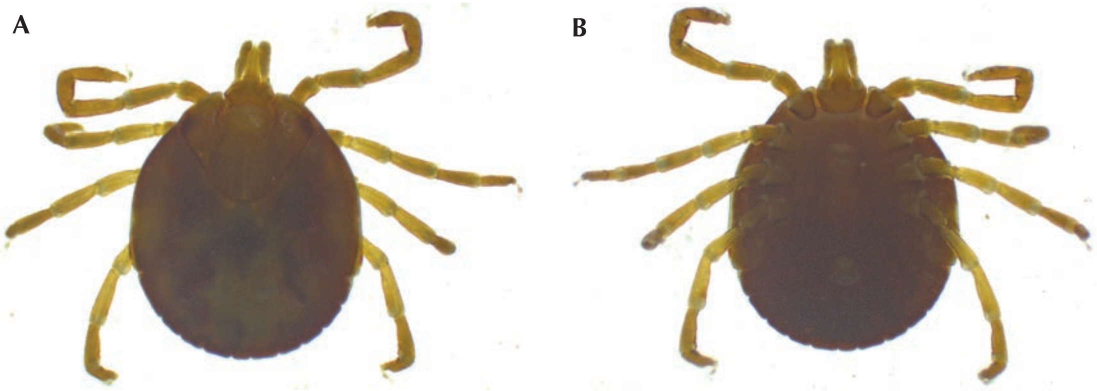

Figure 4. Habitus photos of *Amblyomma nitidum* (♀) collected on *Laticauda laticauda* in northern Taiwan: (**A**) dorsum, (**B**) venter.

(Southcott 1957, Nadchatram 1980, 2006, Takahashi *et al.* 2012). In Taiwan, the only reported sea snake mite to date is the endoparasitic trombiculid *Vatacarus kuntzi* Nadchatram and Radovsky, 1971, which inhabits the respiratory tract (especially the trachea and lungs) and was first described from specimens collected on *L. colubrina* at Orchid Island in 1959 (Nadchatram and Radovsky 1971). Additional fieldwork is recommended to further our understanding of the acarofauna associated with Taiwanese sea snakes.

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SHORT COMMUNICATION

Leptodactylus luctator (Anura: Leptodactylidae) as a host for *Centrorhynchus* sp. (Acanthocephala) cystacanths in the Pampa biome, southern Brazil

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Palavras-chave: Abundância, Acantocéfalos, Hospedeiro paratênico, Intensidade média, Prevalência.

Anurans utilize both aquatic and terrestrial environments. Most species have aquatic larval stages in the form of tadpoles (with herbivorous diets) and adult terrestrial stages (with carnivorous diets based mainly on arthropods) (Duellman and Trueb 1994). These characteristics enable anurans to maintain interactions as both predator and prey with a wide variety of vertebrates and invertebrates throughout the trophic chain, thus helping to regulate biological communities in both terrestrial and aquatic ecosystems (Toft 1980, Kupfer *et al.* 2006, Bernarde 2012, Blanco-Torres *et al.* 2020). Many helminths also spend part of their development in aquatic environments and part in terrestrial environments, a fact that is essential for maintaining their life cycles (Poulin and Morand 2004). Anurans therefore provide a link that enables parasites to make this transition, since both helminths and their vertebrate hosts use the same environments (Aho 1990, Poulin and Cribb 2002).

Anuran amphibians have been associated with a rich diversity of helminths. They act as intermediate, definitive, and paratenic hosts for nematodes, cestodes, digeneans, and acanthocephalans (Campião *et al.* 2014, Fernandes and Kohn 2014, Olivera and Campião 2024, Oliveira *et al.* 2024). Regarding acanthocephalans, adult and infective forms (cystacanths) parasitize anurans in South America, where most reports mention cystacanths of the genus *Centrorhynchus* Lühe, 1911 (Palaeacanthocephala: Centrorhynchidae) associated with species of the anuran families

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Hylidae, Leptodactylidae, and Bufonidae (Campião *et al.* 2014, Olivera and Campião 2024, Oliveira *et al.* 2024; Santos *et al.* 2024). In the Pampa biome (which includes Uruguay, Argentina, and southern Brazil), *Centrorhynchus* cystacanths have been found in *Leptodactylus ocellatus* Girard, 1853 (Leptodactylidae) in Uruguay (Cordero 1933), as well as in species of Hylidae, Bufonidae, and Ranidae in the Brazilian Pampa (Silveira *et al.* 2022, Coimbra *et al.* 2023, Oliveira *et al.* 2024). Anurans play a role in maintenance and transmission of cystacanths to birds and mammals (definitive hosts) through the trophic chain (Petrochenko 1971, Kennedy 2006), since they represent a significant food resource for those predators (e.g. Silva and Talamoni 2003, Porto and Rui 2019, Brentano *et al.* 2020, Frota *et al.* 2021).

The family Leptodactylidae includes species that only occur in the Americas, from southern Texas (USA), southern Sonora (Mexico), and the northern Antilles to southern Brazil, Argentina, and Chile (Frost 2024). *Leptodactylus* Fitzinger, 1826 is comprised of 84 small- to large-sized species (Frost 2024) that may be found in a wide range of environments, including lowland dense rainforest (primary and secondary rainforest), open habitats, disturbed forests, and areas currently used for agriculture and livestock farming (de Sá *et al.* 2014). *Leptodactylus luctator* (Hudson, 1892) occurs from south-central Bahia state and east-central Goiás state to western Mato Grosso state and from southern Brazil, Uruguay, and Paraguay to eastern Bolivia and Argentina (from the south to northern Buenos Aires and southern Córdoba Provinces) (Magalhães *et al.* 2020, Frost 2024). Magalhães *et al.* (2020) provided a taxonomic revision of all species included in the *Leptodactylus latrans* group based on acoustic data, morphological / chromatic variation, and molecular phylogenetics.

Despite many records of *Centrorhynchus* sp. cystacanths in leptodactylid anurans in South America (Campião *et al.* 2014, Olivera and Campião 2024, Oliveira *et al.* 2024, Santos *et al.* 2024), few studies have analyzed infections in relation to body parameters of hosts (Hamann *et al.* 2012). Male and female hosts in species of Bufonidae and Ranidae have similar rates of helminth infections (Santos and Amato 2010a, Coimbra *et al.* 2023, Oliveira *et al.* 2024); however, abundance of cystacanths varied with the snout-vent length of the hosts in different regions (Santos and Amato 2010a, Coimbra *et al.* 2023). Helminthological studies provide data on biodiversity and parasite–host interactions and generate information that may be used by conservation programs. Because the Pampa is considered one of the least protected Brazilian biomes (Ribeiro *et al.* 2021), all knowledge generated in the region provides valuable support for its conservation. This study examined *Centrorhynchus* sp. cystacanths in *L. luctator* and analyzed their infection indices regarding gender, size, and mass of hosts in the Pampa biome, southern Brazil.

Forty-two adult specimens of *L. luctator* (23 females and 19 males) were collected in an urban area with wet, dry, and flooded fields on the west bank where the Laguna dos Patos meets the São Gonçalo Channel in Pelotas (31°46'38.0" S, 52°13'57.2" W), Rio Grande do Sul state, Brazil. Anurans were collected by hand in September ($N = 12$), October ($N = 2$), and December 2017 ($N = 10$), and in April ($N = 17$) and August 2018 ($N = 1$); active searches with the aid of headlamp took place at the beginning of twilight. The frogs were kept in individual plastic containers and taken to the Laboratório de Parasitologia de Animais Silvestres at the Universidade Federal de Pelotas (LAPASIL / UFPel) where they were sacrificed using lidocaine 2% and frozen for later necropsy. Anurans were weighed and measured (snout–vent length, SVL) after thawing, using an electronic precision scale and a digital caliper to the nearest 0.1 mm. Gender was confirmed by analyzing gonads during dissection. The study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO, no. 47397) and by the Ethics and Animal Experimentation Committee at UFPel (CEEA, no. 1859/2015).

During necropsy of the frogs, the following potential sites of infection were examined: oral cavity, esophagus, stomach, small and large intestines, liver, lungs, heart, spleen, pancreas, kidneys, urinary bladder, and coelomic cavity. Helminths were removed from cysts, placed in distilled water, and kept in a refrigerator to induce the proboscis to evert. Subsequently, acanthocephalans were compressed and fixed in AFA (ethanol 70° GL, 93 parts; formalin 37%, 5 parts; glacial acetic acid, 2 parts). Samples were preserved in 70° GL ethanol, stained with Langeron's Carmine, and mounted in Canada Balsam (Amato *et al.* 1991). Acanthocephalans were identified based on Petrochenko (1971), Santos and Amato (2010b) and Oliveira *et al.* (2024). Representative specimens were deposited in the Helminthological Collection at the Oswaldo Cruz Institute (CHIOC 40435, 40436a-b, 40437–40441).

Prevalence (P%), mean intensity of infection (MII), and mean abundance (MA) of helminths were calculated based on Bush *et al.* (1997). P% and MII of cystacanths were compared between the two collection periods (2017 and 2018) and between male and female hosts (using the total sample as well as the two sampling periods). Fisher's Exact test ($p \leq 0.05$) and the bootstrap *t*-test ($p \leq 0.05$) were used to compare P% and MII, respectively, by the Quantitative Parasitology (QPweb) program (Reiczigel *et al.* 2019). The Mann-Whitney *U* test was used to check for differences in body mass (BM) and SVL between males and females. Spearman's correlation coefficient (r_s) was used to estimate the influence of BM and SVL of hosts on the abundance of helminths. Analyses were carried out regardless of the sampling period by Jamovi® version 2.2 (The Jamovi Project 2023).

Thirty-nine individuals (92.9%) were parasitized with cystacanths of *Centhrorynchus* sp. A total of 440 specimens was removed from the coelomic cavity, external surface of the liver, serosa of the stomach, musculature of small and large intestines, and external surface of the urinary system. Mean intensity of infection was 11.3 cystacanths/host (Table 1). Prevalence of *Centhrorynchus* sp. was similar in the two sampling periods (2017 and 2018); however, the MII was significantly ($p = 0.036$) higher in 2018 (16.3 cystacanths/host) compared to the first period (7.41 cystacanths/host) (Table 1). Prevalence and mean intensity of infection in females were 91.3% and 12.8 cystacanths/host, respectively, while in males, values for those parameters were 94.7% and 9.6 cystacanths/host, with no significant intersexual differences (Table 2). Similarly, infections in females and males did not show significant differences between the two sampling periods (Table 2).

Regarding host size, mean SVL of *L. luctator* individuals was 73.6 ± 13.1 mm (range 46.0–102.0 mm) and mean BM was 49.9 ± 23.6 g (range 12.1–113 g). Even though mean SVL of females was significantly ($p = 0.028$) larger than that of males, abundance of cystacanths was significantly correlated only with SVL and BM of males (Table 3).

In South America, records of cystacanths of the genus *Centhrorynchus* infecting species of *Leptodactylus* had lower P% and MII values than those recorded by us in *L. luctator.* These studies revealed P% values ranging from 1.1% to 46.05% and MII between 1.0 and 8.7 cystacanths/host in Argentina (Corrientes, Formosa, and Chaco Provinces; González and Hamann 2006, Hamann *et al.* 2006, Schaefer *et al.* 2006, Hamann *et al.* 2012, Zaracho *et al.* 2012), in Paraguay (Alto Paraguay and Itapua Provinces; Smales 2007) and in Brazil (Ceará state; Oliveira *et al.* 2022). In the Brazilian Cerrado biome, the MII by *Centhrorynchus* cystacanths in *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 and *Leptodactylus vastus* Lutz, 1930 was 4 and 111 helminths/host, respectively; however, only one anuran of each species was parasitized, leading to prevalences of 4% and 7.69%, respectively (Santos *et al.* 2024). Some investigations carried out in the north, central-west, and northeast of Brazil have recorded the occurrence of cystacanths of undetermined genera associated with *Leptodactylus*

Table 1. Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range of *Centrorhynchus* sp. cystacanths parasitizing *Leptodactylus luctator* from the Brazilian Pampa, southern Brazil, in 2017 and 2018. *p-value = 0.036.

Parasitological indexes	**2017 (*N* = 24)**	**2018 (*N* = 18)**	**Total (*N* = 42)**
P%	91.7	94.4	92.9
MII ± SE	7.41 ± 1.37*	16.3 ± 3.45*	11.3 ± 1.81
MA ± SE	6.79 ± 1.32	15.4 ± 3.37	10.5 ± 1.74
Range	1–26	1–46	1–46

Table 2. Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range of *Centrorhynchus* sp. cystacanths parasitizing males and females of *Leptodactylus luctator* from the Brazilian Pampa, southern Brazil, in 2017 and 2018.

Parasitological indexes	**Total**		**2017**		**2018**	
	Females (*N* = 23)	Males (*N* = 19)	Females (*N* = 12)	Males (*N* = 12)	Females (*N* = 11)	Males (*N* = 7)
P%	91.3	94.7	91.7	91.7	90.9	100
MII ± SE	12.8 ± 2.81	9.6 ± 2.16	6.36 ± 1.62	8.45 ± 2.25	19.80 ± 4.82	11.29 ± 4.48
MA ± SE	11.7 ± 2.68	9.1 ± 2.11	5.83 ± 1.57	7.75 ± 2.17	18.0 ± 4.72	11.3 ± 4.48
Range	1–46	1–36	1–20	2–26	3–46	1–36

Table 3. Snout–vent length (SVL) and body mass (BM) of *Leptodactylus luctator* and the Spearman's correlation coefficient (r_s) between *Centrorhynchus* sp. cystacanths abundance and body parameters (SVL and BM) in relation to the total sample of anuran males and females from the Brazilian Pampa, southern Brazil. [a]Mann-Whitney U = 131 (p = 0.028); [b]Mann-Whitney U = 144 (p = 0.061); [c]Significant correlation.

	Total sample (*N* = 42)		**Females (*N* = 23)**		**Males (*N* = 19)**	
	SVL (mm)	BM (g)	SVL (mm)	BM (g)	SVL (mm)	BM (g)
Mean ± SD	73.6 ± 13.1	49.9 ± 23.6	77.6 ± 9.27	55.9 ± 19.83	68.8 ± 15.5	42.7 ± 26.2
Range	46.0–102.0	12.1–112.8	65.0–95.0[a]	29.3–112.8[b]	46.0–102.0[a]	12.1–100.5[b]
r_s	-0.032	-0.065	-0.147	-0.206	0.452	0.436
p-value	0.840	0.682	0.502	0.345	0.030[c]	0.037[c]

spp., also with lower P% (Goldberg *et al.* 2009, Campião *et al.* 2016, Lins *et al.* 2017, Santos *et al.* 2024) than that found in this study. Among the records of cystacanths of undetermined genus, Santos *et al.* (2024) reported an MII (8.27–17 helminths/host) that is similar to the present study, and Lins *et al.* (2017) reported a higher mean intensity (37 cystacanths/host) than that observed in *L. luctator* in the Pampa biome. Cordero (1933) reported *Centrorhynchus tumidulus* (Rudolphi, 1919) cystacanths in *L. ocellatus* in Uruguay; however, the number of hosts examined and the parasite infection indices were not reported. In anurans of the families Bufonidae (Santos and

Amato 2010a, Coimbra *et al.* 2023), Hylidae (Silveira *et al.* 2022), and Ranidae (Oliveira *et al.* 2024) in the Brazilian Pampa, records of cystacanths of *Centhroryynchus* sp. with infection rates were close to those found in this study, i.e., P% ranging between 55.1% and 84% and MII, from 4.79 to 17.1 helminths/host.

The influence of length and body mass of *Leptodactylus bufonius* Boulenger, 1894 on the abundance of *Centrorhynchus* sp. cystacanths was investigated in Argentina (Corrientes Province), where Hamann *et al.* (2012) observed that the larger the anurans, the more cystacanths they bore (considering the total sample, $N = 66$), a fact that was not observed in this study. Analyses of body parameters of males and females have shown that abundance was significantly correlated with SVL in *L. luctator* males (this study) and *L. bufonius* males (Hamann *et al.* 2012); in the case of *L. luctator,* BM and helminth abundance were also correlated. In both species, larger males tend to have a higher abundance of cystacanths, while body parameters of females showed no significant correlation with abundance of parasites (Hamann *et al.* 2012, this study). It should be noted that in *L. bufonius,* males were larger than females (Hamann *et al.* 2012), which is the opposite of *L. luctator.* In other groups of anurans, such as *Rhinella dorbignyi* (Duméril and Bibron, 1841), larger hosts had greater abundances of cystacanths of *Centrorhynchus* sp. (Santos and Amato 2010a, Hamann *et al.* 2013). Larger size is related to longer host life and, consequently, provides more time to be infected, a fact that favors higher parasitism rates (Hamann *et al.* 2013). Oliveira *et al.* (2022) argued that microhabitat use, physiology, behavior, and seasonality may exert more influence on parasite load than anuran body size. Although *L. luctator* males had a higher abundance of cystacanths, there was no difference in P% and MII between males and females, corroborating studies with other anuran species (Santos and Amato 2010a, Coimbra *et al.* 2023, Oliveira *et al.* 2024). In many vertebrate host groups (mainly mammals), the high parasite load in males compared to females has been associated with hormonal and immunological factors (Klein 2004). Lees and Bass (1960) recorded higher parasite loads (including acanthocephalans) in males than in females of naturally infected *Rana temporaria* Linnaeus, 1758. The authors associated this difference with the hormone oestradiol after carrying out an experiment in which the hormone was injected into a group of male *R. temporaria,* which had a drop in parasitism compared to the control group (Lees and Bass 1960). Further studies, not only on cystacanth infections, but also on other groups of helminths associated with *L. luctator,* are needed to assess other aspects of parasite-host interactions.

It is worth mentioning that the diet of anurans influences *Centrorhynchus* infections directly. In general, transmission of *Centrorhynchus* spp. involves prey-predator interactions, i.e., the trophic chain is necessary to develop its life cycle. Cystacanths (infective forms of the parasite) develop in an obligate intermediate host (e.g., isopod crustaceans) and may use facultative paratenic hosts (e.g., amphibians and reptiles) that ingest arthropods and act as carriers of infective forms; birds and mammals (definitive hosts) get infected by ingesting cystacanths while preying on them (Petrochenko 1971, Amato *et al.* 2003, Kennedy 2006). Isopods are part of the diet of *Leptodactylus* spp., which may also prey on other anurans, including other leptodactylids (Teixeira and Vrcibradic 2003, França *et al.* 2004, Maneyro *et al.* 2004, Sanabria *et al.* 2005, Pazinato *et al.* 2011, Solé *et al.* 2019) and thus increase participation of these vertebrates in the *Centrorhynchus* transmission chain. Anurans are valuable food sources for reptiles, birds, and mammals (Panasci and Whitacre 2000, Silva and Talamoni 2003, Aguiar and Di-Bernardo 2004, Hartmann and Marques 2005, Soave *et al.* 2008, Corrêa *et al.* 2016, Thaler *et al.* 2018, Quintela and Loebmann 2019, Porto and Rui 2019, Brentano *et al.* 2020, Frota *et al.* 2021). Adult parasites have been recorded in several species of birds and mammals in South America (Oliveira *et* *al.* 2024), including the Pampa (Cordero 1933, Ruas *et al.* 2008, Gomes *et al.* 2012), reinforcing the idea that the region has favorable conditions for the development and maintenance of the parasites. A diverse network of trophic interactions is involved in the transmission of acanthocephalan parasites. Parasite-host interactions are constantly under the influence of both abiotic (e. g., temperature, humidity, and anthropogenic changes) and biotic factors (e. g., reproductive and physiological aspects of hosts and parasites), which may influence the life cycle of parasites and, as a result, affect parasitism rates.

This is the first record of *Centrorynchus* sp. cystacanths in *L. luctator* in the Brazilian Pampa, where infection indices suggest that this anuran plays a role in maintaining the infective forms, thus contributing to the life cycle of the acanthocephalan. The occurrence of a heteroxenic parasite (i.e., one that necessarily requires more than one host) reinforces the need for conserving ecosystems. Parasites are a natural part of all ecosystems and each species is a potentially useful unit of information (Marcogliese 2005). Helminthological studies carried out in the Pampa biome have helped generate data that may assist in its conservation since it supports complex life cycles.

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SHORT COMMUNICATION

Limb malformations in *Leptodactylus rhodomystax* (Anura: Leptodactylidae) in the Brazilian Amazon

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Palavras-chave: Anfíbios, Anomalias morfológicas, Braquidactilia, Ectrodactilia, Ectromelia, Sindactilia.

Amphibians are among the most threatened vertebrate groups in the world, with 40.7% of species facing some level of extinction risk (Luedtke *et al.* 2023, Toledo *et al.* 2023). The decline in amphibian populations is primarily linked to global climate change, which alters habitats and ecological niches of these species (Luedtke *et al.* 2023). Other factors, such as land use changes, overexploitation, human interference, pollution, and deforestation are interconnected with the loss and degradation of habitats (Stuart *et al.* 2004, Luedtke *et al.* 2023, Toledo *et al.* 2023).

From this perspective, amphibians, which inhabit both aquatic and terrestrial ecosystems throughout their life cycle, are vulnerable to a wide range of biotic and abiotic factors, including water pollution, exposure to toxic chemicals, ultraviolet radiation, parasitic infections, and genetic alterations (Alford and Richards 1999, Ankley *et al.* 2002, Prati *et al.* 2002, Johnson *et al.* 2012, Bessa-Silva *et al.* 2016). Consequently, these factors can lead to morphological anomalies that affect the structure and development of these vertebrates (Henle *et al.* 2017, Souza *et al.* 2021). Such anomalies can range from limb and eye malformations, organ duplications, and other morphological variations (Henle *et al.* 2017, Ajibola and Omoshehin 2024).

Studies on morphological anomalies in anuran amphibians within the Amazon Rainforest are relatively recent and have documented

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malformations across various families, including Brachycephalidae, Bufonidae, Hylidae, Hylodidae, Leptodactylidae, Microhylidae, and Odontophrynidae (Pedroso-Santos *et al.* 2020, Souza *et al.* 2021, Mônico *et al.* 2023). In this study, we report four types of morphological limb malformations in individuals of *Leptodactylus rhodomystax* Boulenger, 1884 from the Brazilian Amazon.

During taxonomic work with specimens of *L. rhodomystax* deposited in the herpetological collection of the Museu Paraense Emílio Goeldi, we identified three individuals exhibiting morphological limb malformations. We captured images of these specimens and described the malformations using the terminology adopted by Meteyer (2000) and Henle *et al.* (2017).

The first specimen (MPEG25877 ♂; SVL: 61.89 mm; Figure 1A–B), collected in March 2008 at UHE Belo Monte, Vitória do Xingú municipality, Pará state (03°07'01.14" S, 51°47'35.51" W; WGS 84), exhibited tibial-fibular ectromelia in the right hind limb (Figure 1A–B). This condition was characterized by an incomplete limb with the absence of the lower part of the leg. Due to the lack of radiographs, we could not confirm whether the metatarsal and phalangeal bones were fused in the terminal part of the affected limb.

The second specimen (MPEG36537 ♂; SVL: 66.65 mm; Figure 1C–D), collected in May 2012 at Feijó municipality, Acre state (08°08'35.82" S, 70°20'36.43" W; WGS 84), exhibited a combination of ectrodactyly, brachydactyly, and syndactyly. These conditions were characterized by the absence of the fourth digit, shortening of the third digit, and fusion of the second and third digits of the left forelimb, respectively.

The third specimen (MPEG29190 ♂; SVL: 71.82 mm; Figure 1E), collected in February 2006 at Parque Ecológico do Gunma, Santa Bárbara do Pará municipality, Pará state (01°13'02.24" S, 48°17'40.07" W; WGS 84), exhibited ectrodactyly, characterized by the complete absence of the second digit of the left forelimb (Figure 1E).

Limb malformations are among the most frequently reported anomalies in anurans (Correia *et al.* 2018, Ascoli-Morrete *et al.* 2019, Souza *et al.* 2021, Gobel *et al.* 2022, Baroni *et al.* 2024). Although the etiology of these malformations remains unclear, studies suggest that the affected regions may be directly associated with specific teratogenic factors (Gardiner and Hoppe 1999, Lannoo 2009). Some authors propose potential connections between trematode infections, UV-B radiation, and exposure to agricultural chemical contaminants with the occurrence of limb abnormalities (Gardiner and Hoppe 1999, Spolyarich *et al.* 2011, Johnson *et al.* 2012). Furthermore, species with terrestrial and semiaquatic habits, such as those in the genus *Leptodactylus,* may exhibit a higher prevalence of malformations affecting locomotor limbs compared to arboreal species (Peltzer *et al.* 2011, Agostini *et al.* 2013, Gobel *et al.* 2022). These authors suggest that such results may be linked to varying survival rates of abnormal individuals in different microhabitats. The malformations found in terrestrial species may not significantly impact survival rates (Baroni *et al.* 2024); in fact, all individuals analyzed in this study are adults. Nevertheless, these malformations may negatively influence the reproductive success of these animals, emphasizing the need for further studies on this topic (Baroni *et al.* 2024). The impact of these anomalies may differ in arboreal species, given their greater reliance on specific motor skills for locomotion and survival in these habitats (Goodman and Johnson 2011, Agostini *et al.* 2013).

Records of morphological anomalies in Brazilian anurans are frequently reported for the family Hylidae, followed by the family Leptodactylidae (Souza *et al.* 2021). For the genus *Leptodactylus,* anomalies have been described in various species, including *L. fuscus* (Schneider, 1799) (Borges *et al.* 2019), *L. latinasus* Jiménez de la Espada, 1875 (Gobel *et al.* 2022), *L. latrans* (Steffen, 1815) (Carezzano *et al.* 2016), *L. longirostris* Boulenger, 1882

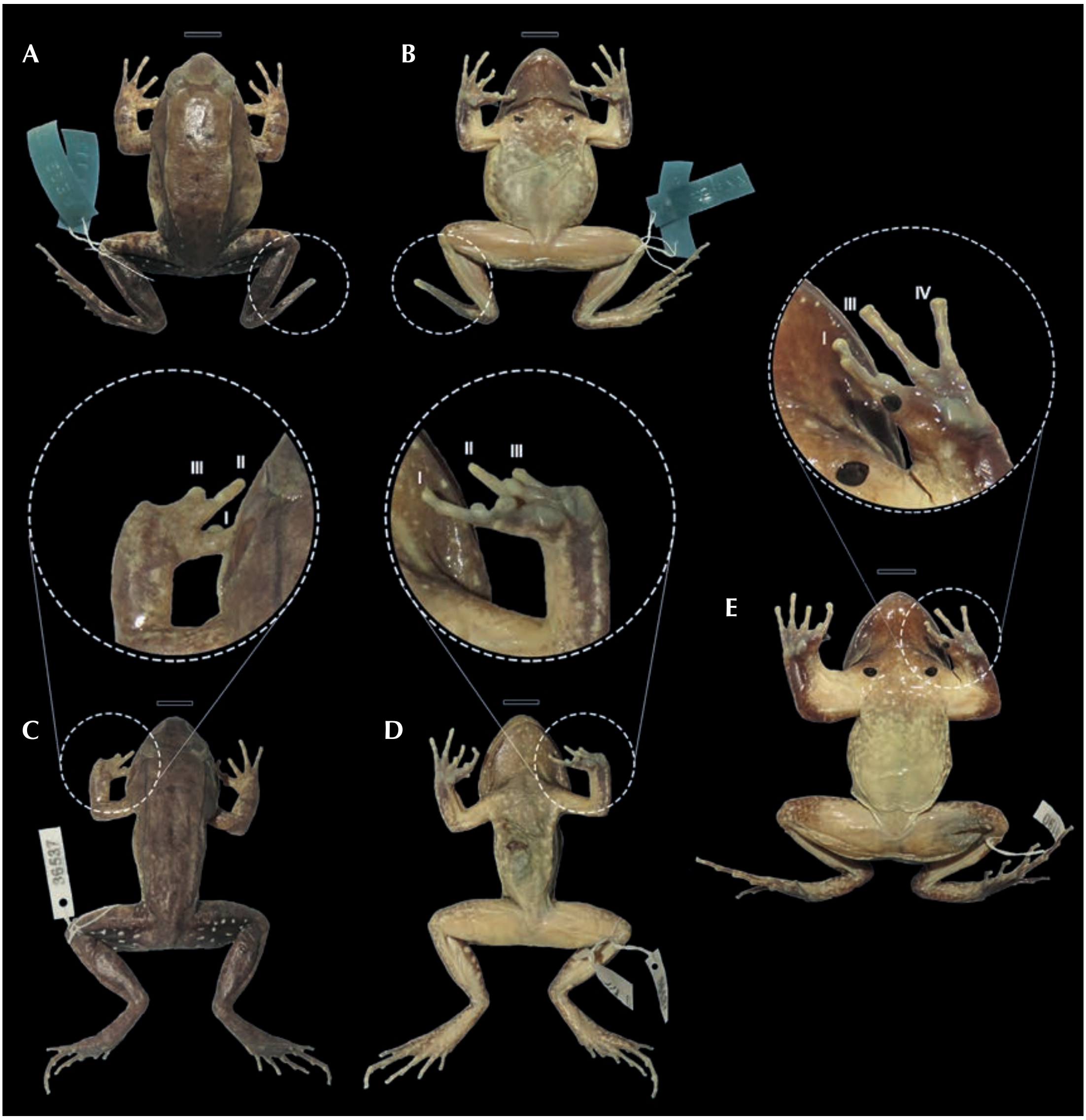

Figure 1. Limb malformations in *Leptodactylus rhodomystax* from the Brazilian Amazon. Specimen MPEG25877 showing tibial-fibular ectromelia in the right hind limb, with dorsal (**A**) and ventral (**B**) views. Specimen MPEG36537 exhibiting ectrodactyly, brachydactyly, and syndactyly in the left forelimb, with dorsal (**C**) and ventral (**D**) views. (**E**) Specimen MPEG29190 displays ectrodactyly of the second digit of the left forelimb. Scale bar = 10 mm.

(Pedroso-Santos *et al.* 2020), *L. luctator* (Hudson, 1892) (Gobel *et al.* 2022), *L. mystaceus* (Spix, 1824) (Pedroso-Santos *et al.* 2020), *L. mystacinus* (Burmeister, 1861) (Ascoli-Morrete *et al.* 2019), *L. petersii* (Steindachner, 1864) (Pedroso-Santos *et al.* 2020), *L. podicipinus* (Cope, 1862) (Pedroso-Santos *et al.* 2020), *L. troglodytes* Lutz, 1926 (Santos and Silva 2010), and *L. vastus* Lutz, 1930 (Braga *et al.* 2013). Among the main types of morphological malformations are those associated with locomotor limbs, such as brachydactyly, ectrodactyly, and ectromelia (Souza *et al.* 2021, Gobel *et al.* 2022), which are consistent with our findings for *L. rhodomystax.* It is important to mention that the high prevalence of limb malformations may be related to the limitations of methods that analyze only external morphology. The application of complementary techniques, such as radiography, becomes crucial for a more comprehensive understanding of the anomalies present in the internal morphology of these organisms (Peltzer *et al.* 2011, Baroni *et al.* 2024).

Although the causes of the malformations in the examined specimens were not identified, these reports represent the first record of morphological malformations in *L. rhodomystax.* They provide new insights into the occurrence of such anomalies among Amazonian amphibians and contribute to a better understanding of potential geographic and taxonomic patterns of this phenomenon.

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Editor: Tamí Mott

INSTRUCTIONS TO AUTHORS

General Information. *Phyllomedusa* publishes articles dealing with the entire field of herpetology. The journal also maintains sections for Short Communications and Book Reviews. Manuscripts are considered on the conditions that they: (1) have not been published elsewhere; (2) are not under consideration for publication, in whole or in part, in another journal or book; and (3) are submitted by the authors in the format and style of *Phyllomedusa* and in accordance with the specifications included in the Instructions to Authors. Manuscripts should be submitted as a single Microsoft Word document via e-mail. High-quality color images are accepted. Manuscripts must be written in English with appropriate abstracts in alternate languages. If English is not your primary language, arrange to have your manuscript reviewed for English usage before you submit it. Direct any questions about manuscript submission to the primary editor. Publication in *Phyllomedusa,* including color images, is free of charge.

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Results
Discussion
Acknowledgments
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Vanzolini, P. E. 1993. A new species of turtle, genus *Trachemys,* from the state of Maranhão, Brazil (Testudines, Emydidae). *Revista Brasileira de Biologia 55:* 111–125.

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✓ **More than two authors in a journal series:**

Hero, J.-M., W. E. Magnusson, C. F. D. Rocha, and C. P. Catterall. 2001. Antipredator defenses influence the distribution of amphibian prey species in the central Amazon rain forest. *Biotropica 33:* 131–141.

✓ **Chapter in an edited volume:**

Hedges, S. B. 1999. Distribution patterns of amphibians in the West Indies. Pp. 211–254 *in* W. E. Duellman (ed.), *Patterns of Distribution of Amphibians. A Global Perpective.* Baltimore and London. The Johns Hopkins University Press.

✓ **Unpublished thesis or dissertation:**

Verdade, V. K. 2001. Revisão das espécies de *Colostethus* Cope, 1866 da Mata Atlântica (Anura, Dendrobatidae). Unpublished M.Sc. Dissertation. Universidade de São Paulo, Brazil.

✓ **Book:**

McDiarmid R. W. and R. Altig (eds.). 1999. *Tadpoles. The Biology of Anuran Larvae.* Chicago and London. The University of Chicago Press. 633 pp.

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Frost, D. R. (ed.). 2010. Amphibian Species of the World: an Online Reference. Version 5.4 (8 April 2010). Electronic Database accessible at http: // research.amnh.org/vz/herpetology/amphibia/American Museum of Natural History, New York, USA. Captured on 22 August 2010.

✓ **Software:**

Maddison, W. P. and D. R. Madison. 2010. Mesquite. A Modular System for Evolutionary Analysis. Version 2.73. URL: http://mesquiteproject.org

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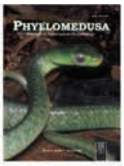
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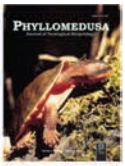
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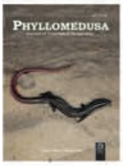
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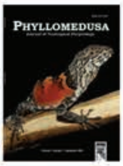
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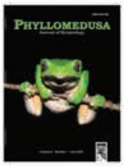
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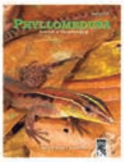
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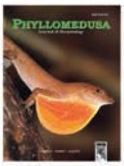
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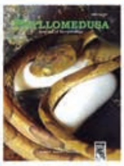
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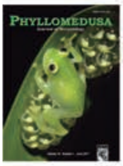
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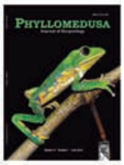
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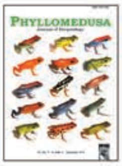
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