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Lagartixa exótica *Lepidodactylus lugubris*

Boletim do Museu Paraense Emílio Goeldi

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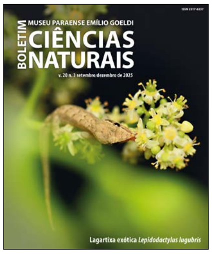

IMAGEM DA CAPA
Lagartixa *Lepidodactylus lugubris* alimentando-se de néctar das flores de cajá-manga (*Spondias dulcis).*
Foto: C. A. C. Favacho (2017).

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CARTA DO EDITOR

O sumário da última edição de 2025 do **Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais** é composto por manuscritos distribuídos nas áreas de ciências da terra, paleontologia e zoologia, refletindo a diversidade temática e metodológica da revista e reafirmando seu compromisso com a qualidade editorial e a valorização da pesquisa em ciências naturais.

O primeiro artigo do sumário, de autoria de Menezes e colaboradores, analisa a variação da linha costeira no município de Soure, ilha do Marajó, na Amazônia paraense, ao longo de um período médio de quase cinco décadas (1972-2020), a partir de técnicas de sensoriamento remoto e do uso do *Digital Shoreline Analysis System* (DSAS). Com base em imagens Landsat e na aplicação de índices e métricas consagradas, o estudo demonstra a predominância de processos erosivos em parte significativa da área analisada, ao mesmo tempo em que identifica setores de acreção. Os resultados oferecem subsídios técnicos relevantes para o entendimento da dinâmica costeira estuarina e para o planejamento de medidas de gestão e mitigação em regiões costeiras amazônicas.

O artigo de Cardoso e Gorayeb apresenta um estudo detalhado sobre os estágios imaturos da mosca-da-madeira (nome científico: *Pantophthalmus kerteszianus*; família: Pantophthalmidae), definindo, pela primeira vez, o número total de ínstares larvais da espécie com base em análises morfológicas e biométricas. O trabalho descreve o segundo ínstar larval e redescreve o último ínstar e a pupa, a partir de material coletado em árvores de paricá, no município de Paragominas, Pará. Além de estabelecer que a espécie possui sete ínstares larvais, o estudo fornece descrições minuciosas, ilustrações e comparações com espécies congêneres, representando uma contribuição relevante para o conhecimento da biologia e da morfologia de Pantophthalmidae na região Neotropical.

Chupil e colaboradores trazem um estudo detalhado sobre a ecologia reprodutiva e o comportamento de espécies de aves das famílias Ardeidae e Threskiornithidae em uma colônia mista, localizada em um estuário tropical do litoral sul do estado de São Paulo. Com base em um monitoramento feito ao longo de três estações reprodutivas, o trabalho descreve padrões de ocupação espacial, dinâmica temporal da reprodução e interações comportamentais entre seis espécies, com destaque para o papel do guará (*Eudocimus ruber*) na organização da colônia. Os resultados reforçam a importância de estudos de longa duração para a compreensão da dinâmica reprodutiva de aves aquáticas coloniais e fornecem subsídios relevantes para sua conservação em ambientes estuarinos.

O sumário desta edição é composto também por cinco notas científicas. A primeira nota, de autoria de Nayara e colaboradores, amplia de forma significativa o conhecimento sobre a distribuição geográfica de duas espécies de peixe da família Acestrorhamphidae (nomes científicos: *Astyanax courensis* e *Psalidodon goyanensis*), na bacia do alto rio Tocantins. Ao documentar novos registros em tributários do Distrito Federal e de Goiás, inclusive em áreas protegidas, o estudo reforça a importância das coleções científicas e de levantamentos regionais para a compreensão da biodiversidade do Cerrado, além de oferecer subsídios relevantes para a sistemática, a biogeografia e a conservação da ictiofauna neotropical.

A nota de Carvalho-Filho e Favacho documenta o primeiro registro da lagartixa exótica *Lepidodactylus lugubris* no estado do Amazonas e detalha aspectos de sua biologia alimentar em ambientes urbanos da cidade de Belém, Pará. Diversos espécimes foram observados alimentando-se de néctar de espécies vegetais nativas e exóticas em jardins e quintais, bem como de frutos (banana e abacaxi) e produtos industrializados açucarados. Além de ampliar a distribuição conhecida da espécie no Brasil, o estudo evidencia sua notável plasticidade ecológica e capacidade de explorar recursos antrópicos, características que reforçam seu potencial invasor.

Lasmar e colaboradores apresentam novos registros do tamanduá-bandeira (nome científico: *Myrmecophaga tridactyla*) na região sul do estado de Minas Gerais, espécie atualmente classificada como vulnerável em níveis regional, nacional e global. A partir de dados obtidos por observação direta e armadilhas fotográficas, o estudo documenta duas novas localidades nos municípios de Lavras e Santo Antônio do Amparo, ampliando o conhecimento sobre a distribuição geográfica da espécie em uma área de ecótono entre Cerrado e Mata Atlântica. Esses registros são relevantes para o entendimento da dinâmica espacial da espécie e fornecem subsídios importantes para ações de monitoramento e conservação.

Moraes-Santos e colaboradores trazem contribuição relevante ao conhecimento da paleontologia amazônica ao apresentar uma nova ocorrência de sirênio – grupo que inclui os peixes-boi – na Formação Pirabas, no Mioceno da Amazônia oriental. O material consiste em costelas e partes cranianas, incluindo um rostro fragmentado (pré-maxilar com restos de dente incisivo), coletado na praia de Fortalezinha, ilha de Maiandeua, município de Maracanã, e depositado na coleção paleontológica do Museu Paraense Emílio Goeldi. Ao apresentar material fossilífero que inclui raros elementos cranianos, o estudo amplia o registro desses mamíferos aquáticos no Norte do Brasil e contribui de forma significativa para o entendimento da diversidade, da paleobiogeografia e da evolução dos sirênios na América do Sul durante o Neógeno.

A nota de Andrade e colaboradores apresenta um método eficiente e de baixo custo para a amostragem de visitantes florais em flores pendentes, tendo como modelo a espécie de planta *Clusia grandiflora* (Clusiaceae). O estudo propõe o uso de uma haste entomológica adaptada com tubo Falcon, permitindo a captura direta de insetos em flores voltadas para baixo, sem a necessidade de podas ou movimentos bruscos. Testado no Parque Nacional dos Lençóis Maranhenses, o método demonstrou alta eficiência, praticidade e potencial para complementar técnicas tradicionais em estudos de ecologia da polinização e biologia reprodutiva de plantas.

Para encerrar esta carta, reitero meus agradecimentos aos avaliadores de diversas instituições do Brasil, pela valiosa dedicação à leitura crítica e à avaliação dos manuscritos publicados nesta edição. Agradeço, igualmente, aos editores de seção pelo criterioso e cuidadoso trabalho de editoração dos artigos aceitos: Dra. Alexandra Maria Ramos Bezerra, Dr. Alexandre Felipe Raimundo Missassi, Dra. Débora Rodrigues Souza-Campana, Dr. Leonardo de Sousa Miranda, Dra. Milena Marília Nogueira de Andrade e Dra. Valéria Juliete Silva. Estendo ainda meu reconhecimento a Diana Gomes Lopes, Júlia Modesto, Rafaele Lima e Talita do Vale, pelo empenho, profissionalismo e atenção dedicados às etapas editoriais.

Fernando da Silva Carvalho Filho
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CARTA DO EDITOR
EDITOR'S NOTE

ARTIGOS CIENTÍFICOS
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Artigos Científicos

Variation of the coastline in a medium period (1972-2020): An analysis in the municipality of Soure, *paraense* Amazon

Variação da linha costeira em um médio período (1972-2020): uma análise no município de Soure, Amazônia paraense

Rafael Alexandre Alves Menezes[I] | Diandra Karina Martins Guimarães[I] | Maamar El Robrini[I]

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Abstract: Estuarine erosion is a significant environmental issue that affects various coastal regions around the world. The western coast of the Pará River estuary mouth is located in the municipality of Soure, in the state of Pará. To analyze the multitemporal variability of erosive and/or accretion rates of the coastline (CL), remote sensing techniques and the Digital Shoreline Analysis System (DSAS) were employed. Based on orbital imagery from the Landsat sensors (MSS, TM, OLI) from 1972 to 2020, the CL for each year was detected using the Modified Normalized Difference Water Index (MNDWI). This approach enabled the creation of transects from the onshore baseline and the quantification of NSM, EPR, and LRR rates, processed using DSAS v5. The results indicated that erosion is predominant in 57% of the transects, while accretion was observed in 43%, with an average erosion rate of –2.1 m/year (Sector I–SE) and an average accretion rate of 2.55 m/year (Sector II–NE). The results obtained through remote sensing techniques generated substantial data on the dynamics of the CL, corroborating essential technical procedures for implementing mitigation measures in the region and in other geographical areas.

Keywords: DSAS v5. Estuarine dynamics. Erosion. GIS. Remote sensing.

Resumo: A erosão estuarina é um problema ambiental significativo que afeta várias regiões costeiras ao redor do mundo. A costa oeste da foz do estuário do rio Pará se localiza no município de Soure, no Pará. Para analisar a variabilidade multitemporal das taxas erosivas e/ou a acreção da linha costeira (LC), foi utilizado o sensoriamento remoto e o *Digital Shoreline Analysis System* (DSAS). A partir de imagens orbitais dos sensores Landsat (MSS, TM, OLI) de 1972 a 2020, a LC de cada ano foi detectada utilizando o índice da diferença de água normalizada modificado (MNDWI). Essa abordagem permitiu a criação de transectos a partir da linha de base *onshore*, e a quantificação das taxas de NSM, EPR e LRR, processadas via DSAS v5. Os resultados mostraram que a erosão é predominante em 57% dos transectos, enquanto a acreção foi detectada em 43% dos transectos, sendo que a taxa média de erosão foi de -2,1 m/ano (setor I-SE) e a taxa média de acreção foi de 2,55 m/ano (setor II-NE). Os resultados obtidos através das técnicas de sensoriamento remoto geraram dados substanciais sobre a dinâmica da LC, corroborando procedimentos técnicos essenciais para implementar medidas de mitigação na região e em outros espaços geográficos.

Palavras-chave: DSAS v5. Dinâmica estuarina. Erosão. GIS. Sensoriamento remoto.

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INTRODUCTION

The Coastal Zone (CZ) refers to the geographic area of interaction between the air, sea, and land, including its renewable and non-renewable resources, encompassing both a maritime and a terrestrial strip (Baral et al., 2018). The former refers to the area extending twelve nautical miles from the baseline (low-water line), thus encompassing the entirety of the territorial sea. The latter is the area within the boundaries of municipalities affected by urbanization, port activities, tourism, and industrial activities (MMA, 2018).

The coastline (CL) is used as a geomorphological indicator of coastal dynamics due to its position being variable in time and space (Bertacchini, 2010; Jana et al., 2014; Mahapatra et al., 2014b; Ding et al., 2019). Its movement inland reflects retreat or erosion (Baral et al., 2018). Conversely, its movement towards the offshore or the bay represents advance or accretion (Silva et al., 2016). These changes occur over a medium (~100 years) period and can be characterized mainly through the analysis of data available from orbital sensors (França, 2003). The main factors acting on the CZ are: rainfall, winds, waves, tidal currents, sediment transport and deposition, leading to constant modifications of the CL (Kannan et al., 2014; Wang et al., 2014; Ahmed et al., 2021).

Remote sensing through CL detection technologies is frequently used to provide information about the morphology of the CZ. Thus, the mosaic of satellite images and the vectorization of the CL emerge as a way to identify variations occurring in the CZ (França, 2003; Genz et al., 2007; S. Rodrigues & Souza Filho, 2011; Mahapatra et al., 2014a; Luijendijk et al., 2018; Mentaschi et al., 2018; Orlando et al., 2019; Muskananfola et al., 2020). For the evaluation and quantification of erosion and accretion rates, the Digital Shoreline Analysis System (DSAS) applied to ArcGIS software (Starting from the ArcView GIS 3.x version) assists in determining the variations occurring in the CL over time and space, and is frequently used by the scientific community to analyze the dynamic fluctuations occurring on coasts worldwide (Farias & Maia, 2010; Conti & M. Rodrigues, 2011; Ranieri & El-Robrini, 2015; Mahapatra et al., 2014a; Himmelstoss et al., 2018; Galvez et al., 2020; C. A. G. Santos et al., 2021b).

The dynamics of the CL necessitate the identification and study of different aspects over various temporal perspectives, encompassing short, medium, and long-term spatialization (Tran Thi et al., 2014; Bheeroo et al., 2016; Chenthamil Selvan et al., 2016; Baral et al., 2018). For this purpose, the acquisition of satellite images and the utilization of Geographic Information System (GIS) environments provide a way to understand the dynamics of the LC over time. This includes data that can be found in numerous databases, allowing for the analysis of a large portion of space with satisfactory precision for the analysis component. Consequently, residual errors are minimized, resembling in situ approaches (Alesheikh et al., 2007; Al-Hatrushi, 2013; Esmail et al., 2019; C. A. G. Santos et al., 2021b).

Furthermore, the study of coastal geomorphology has an improvement configuration for the current scenario, a fact that is linked to the use of GIS (Mcfadden et al., 2007; Kaliraj et al., 2015; Li et al., 2015) and the use of the range of satellite images available from the United States Geological Survey (USGS) (França & Souza Filho, 2003; Mahapatra et al., 2014a; Souza Filho, 2005; Santos, 2017). The multitemporal variability of CL erosion or advance rates can be quantified using geospatial techniques, and the analysis of these data is used to understand the dynamics of the CZ, quantifying the rates of variables over time and space (Himmelstoss et al., 2018; Stanchev et al., 2018; Esmail et al., 2019; Nassar et al., 2019; Muskananfola et al., 2020; L. Lima et al., 2021; Mishra et al., 2022; Quadrado et al., 2021).

Many studies in the Brazilian CZ have been published in recent years on coastal analysis, each with its own temporal/spatial particularities and distinct variabilities (Silva et al., 2016; Duarte et al., 2018; Aquino da Silva et al., 2019; Carvalho et al., 2020). However, there are few studies focusing on LC dynamics in the Amazon, particularly in the state of Pará (Ranieri & El-Robrini, 2015; Baía et al., 2021).

Therefore, the study conceived from LC analysis, using satellite images from different years (through overlaying visually interpreted vectors) as the main method to identify variations in the Pará CZ (França, 2003; S. Rodrigues & Souza Filho, 2011). In this perspective, besides direct vector overlay and segmentation, the DSAS tool in ArcGIS software contributed to understanding the multitemporal variations occurring in the coastal scenario of Pará (Conti & M. Rodrigues, 2011; Ranieri & El-Robrini, 2015; Baía et al., 2021).

CZ of the largest fluvial-marine island on the planet (Marajó Island, Pará) is influenced by two major estuaries, those of the Amazon and Pará rivers. These are subject to constant morphological changes, imparting to the coast a dynamic due to its geographical position. The study area is in the eastern part of Marajó Island (Pará, 2020), influenced by the waters of the Pará River estuary, with physical processes at micro and mesoscale generated by forcings from different sources (astronomical tides and river discharge) (Prestes, 2016; Prestes et al., 2014, 2017, 2020). This shoreline comprises the mangrove belt known as the Macromare Mangrove Coast of the Amazon (CMMA), spanning 650 km along the coast from Marajó Bay (Pará) to Tubarão Point (Maranhão) (Souza Filho, 2005), forming part of the largest continuous mangrove belt on the planet, underscoring the importance of understanding coastal processes in the region.

Determining the multitemporal behavior of the CL systematically contributes to integrated coastal management (Pessoa et al., 2019). However, evaluating the evolutionary scenarios of the CZ becomes challenging, as they are subjected to hydrodynamic forces (waves, tides, sea level oscillation), neotectonics, climate (winds, rainfall), and anthropogenic factors (civil and private constructions, deforestation) (Boye et al., 2018; Ataol et al., 2019; Mishra et al., 2022; Ahmed et al., 2021; C. A. G. Santos et al., 2021a), which condition the geographical space of analysis with difficult-to-measure particularities. In some cases, natural dynamic vectors modify the CZ to extents unimaginable when compared to anthropogenic modifications. In this context, the study area corresponds to the western portion of the Pará River estuary (eastern shore of the municipality of Soure, Pará), where natural dynamism prevails, while anthropogenic influences are concentrated in the southeastern fragment of Soure municipality.

Based on the above, this manuscript aims to analyze the evolution of the CL in the western portion of the Pará River Estuary mouth from 1972 to 2020 (constrained by the available Landsat historical series), utilizing satellite imagery, geoprocessing techniques, and leveraging the DSAS v5 plugin to quantify and analyze changes over the proposed 48-year temporal scenario. Thus, the study seeks to comprehend erosive and accretionary dynamics occurring at this temporal scale and to examine the hydrographic aspects influencing the CL. This study focuses on a specific scenario compared to other CZs worldwide, prominently situated in the Brazilian Amazon, characterized by unique processes due to its geographical location and structural morphogenesis (hydrology, climate, geology, and geomorphology).

The generated results are of paramount importance for understanding estuarine dynamics and are expected to contribute to capacity development activities that can provide responses to policymakers and the local, national, and global society. The techniques and data measurements used can be reproduced comprehensively and coherently. Furthermore, this work aims to contribute to the studies in the Decade (2020-2030) of Ocean Science, particularly to United Nations Sustainable Development Goal 13, aiming to contribute to the reduction/mitigation of global changes.

MATERIALS AND METHODS

STUDY AREA

The study area encompasses the CL of the municipality of Soure, located in the mesoregion of Marajó (microregion of Arari), bounded by: NW: 0° 14’ 3.23” S and 48° 59’ 39.65” W; NE: 0° 14’ 59.18” S and 48° 22’ 0.99” W; SW: 0° 41’ 58.61” S and 48° 59’ 10.02” W; SE: 0° 51’ 29.08” S and 48° 22’ 16.88” W (Figure 1).

Figure 1. Location map of the Municipality of Soure, Marajó Island, 2024. Source: IBGE (2021), USGS (2018), ANA (2021).

This area belongs to the geomorphological unit of the Marajoara Gulf, characterized by estuarine and deltaic elements, forming an extensive Quaternary sedimentary basin with kilometric thickness, controlled by strike-slip faults that guide the mouth of the Amazon River. This unit is subdivided into two other geomorphological units: Fluvio-marine Plains and Low Amazon River Plateaus, which encompass a range of depositional environments of fluvial, marine, lacustrine, or mixed origin (Melo & Simões, 2016). On the eastern shore, the structure corresponding to Barreiras sediments is composed of sandstones and claystones, followed by Post-Barreiras sediments (Rossetti et al., 2008a, 2008b) (Figure 2).

Soure is climate is hot and humid, characterized by its proximity to the Atlantic Ocean, influenced by maritime conditions and its location near the equator, resulting in abundant solar energy. From December to May, Soure experiences the rainy season due to the influence of both the warm Atlantic Equatorial and Continental air masses, occasionally intersected by cold Atlantic polar air masses, which enhance precipitation. From June to November, the region experiences the dry season, with higher temperatures. The average annual temperature ranges between 24 °C and 32 °C (INMET, n.d.).

The winds in Soure reach speeds between 6.2 m/s and 7 m/s (INMET, n.d.), predominantly from the northeast direction due to the movement of the Intertropical Convergence Zone (ITCZ). Wind speeds can vary in intensity between the western and eastern sectors due to topographical differences. Vegetation cover

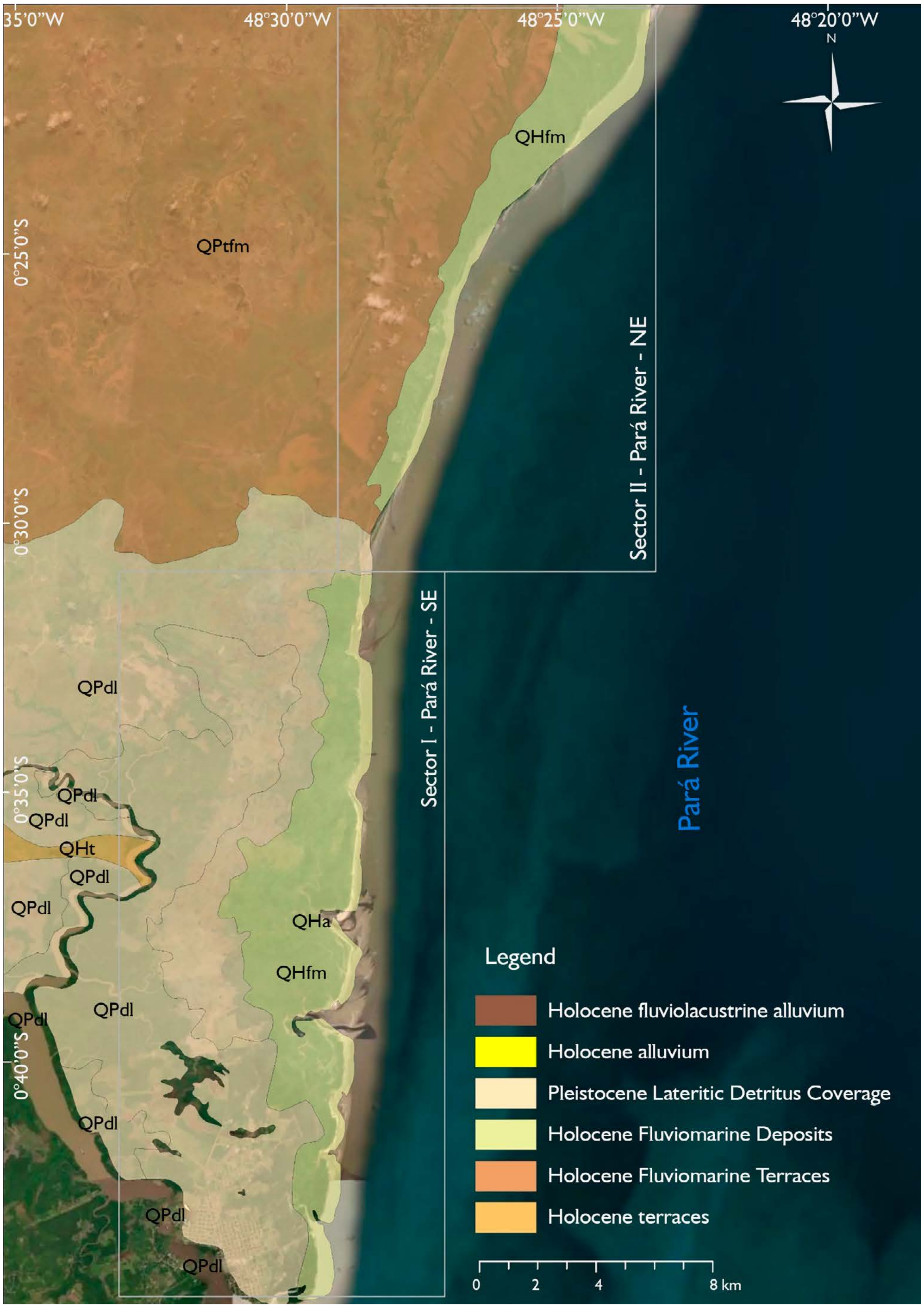

Figure 2. Geological map of the eastern shore of Marajó Island, 2024. Legends: QHfm = Holocene fluviomarine deposits, QPtfm = Pleistocene fluviomarine terraces, QPdl = Pleistocene lateritic detritus cover, QHt = Holocene terraces, QHa = Holocene alluvium. Source: Lacerda et al. (2008).

also influences wind patterns, as forests in the CZ act as natural barriers to winds coming from the northeast toward the eastern coast, significantly reducing their speed (A. Lima et al., 2005).

The eastern coast of Soure is influenced by the hydrodynamics of the Pará River, experiencing a meso to macrotidal regime (França & Souza Filho, 2003). The river flow rates are 98,594 m^3/s during the rainy season and 65,269 m^3/s during the dry season (El-Robrini et al., 2018). Tidal heights range from 5.6 m (high tide/spring tide) to 0.4 m (low tide/neap tide) (Marinha do Brasil, n.d.), and tidal currents reach speeds of 1.2 m/s (flood tide) and 1.4 m/s (ebb tide) (Rosário et al., 2016).

METHODOLOGY

The methodological procedure used to assess coastal erosion in Soure municipality, Pará, involved geospatial techniques in an office setting, utilizing data obtained from the Digital Shoreline Analysis System (DSAS) version 5 tool. The workflow is illustrated in Figure 3.

The process of selecting the projection system used in this study involves the prerequisite of quantifying the data to be generated and utilizing cartographic bases in reference systems with Universal Transverse Mercator (UTM) projections. Therefore, the chosen projection system uses meters (m) as the unit of measurement to calculate distances and project the position of specific components, providing quantitative data relevant to

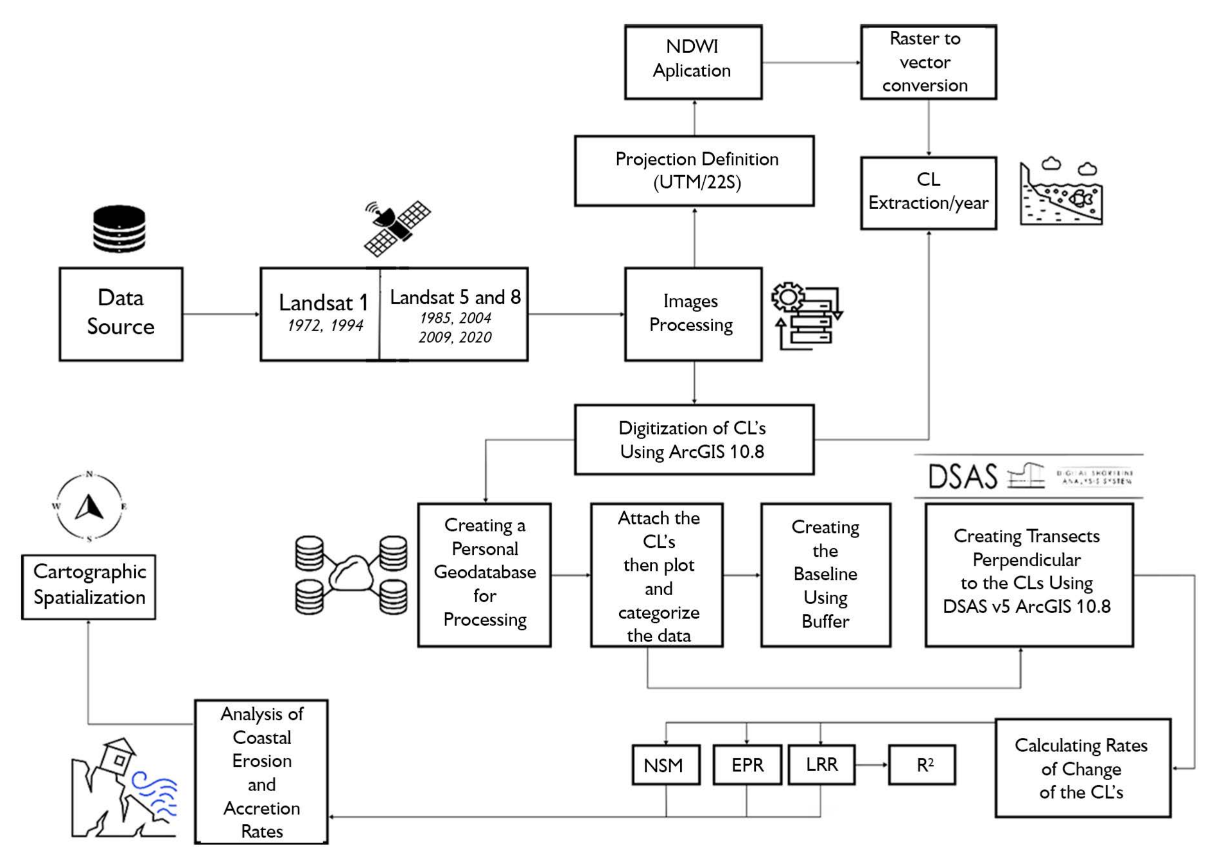

Figure 3. Methodological flow of coastal erosion analysis using DSAS v5. Organization: Author (2024).

the area in question. Additionally, the DSAS v5 plugin, employed in ArcGIS 10.8 software (accessed at the Geographic Information Lab - LAIG/UFPA), is based on the use of UTM coordinate system for interpolation within the GIS environment and quantification of data generated post-processing. If another type of projection were used, it would result in processing errors and prevent the generation of final data. Due to the area's large extent (~60 km), the study area is divided into two sectors: I) Rio Pará - SE and II) Rio Pará – NE (Figure 2).

ACQUISITION OF ORBITAL IMAGES

To ensure the execution of the analysis of CL variation in the areas influenced by the Pará River estuary, images were selected for comparison. These include Landsat 1 MSS medium-resolution images from 1972 and 1994, Landsat 5 TM images from 1985, 2004, and 2009 with a spatial resolution of 30 m, and Landsat 8 OLI images from 2020 with a spatial resolution of 15 m after merging band 8 (panchromatic). These images were obtained from the United States Geological Survey (USGS) website, accessed in September 2020 (Table 1). The selection of these image datasets was based on their minimal cloud cover (< 30%) over the coastal CL vectorization area.

CALCULATION OF GEOMETRIC ERRORS IN THE IMAGES

To ensure data reliability and result accuracy, a cumulative geometric error was defined among the images used. Considering the georeferencing error of each image extracted from the metadata and relating it proportionally to the pixel value, an error of 56.688 m was obtained, averaging 9,478 m annually for linear measurements and an error of 1,704.6 m^2, with 284.1 m^2 for polygonal area measurements (Table 2).

The calculated errors serve as control parameters for measuring the generated data, with the main focus on

Table 1. Satellite images and optical sensors used in processing. Source: USGS (2018). Organization: Author (2024).

Satellite	Optical sensor	Orbit point	Acquisition date	Climatic parameter	Hour	Spatial resolution (m)
Landsat 1	MSS	240060	13/12/1972	El Niño	12:56	80
Landsat 5	TM	224060	09/10/1985	No ocurrence	12:57	30
Landsat 1	MSS	224060	30/07/1994	No ocurrence	12:46	30
Landsat 5	TM	224060	23/06/2004	No ocurrence	13:10	30
Landsat 5	TM	224060	09/09/2009	El Niño	13:18	30
Landsat 8	OLI	224060	07/09/2020	La Niña	13:29	15

Table 2. Geometric errors in the images. Organization: Author (2024).

Year of image acquisition	Pixel error	Linear geometric error (m)
1972	0.370	11,124
1985	0.326	9,781
1994	0.384	11,523
2004	0.310	9,304
2009	0.352	10,573
2020	0.152	4,563
Accumulative geometric errors		56,868
Average errors		9,478

correlating them with the Coastal Net Shore Movement (NSM) data.

VECTORIZATION OF THE CL

The vectorization of CL was created using semi-automatic methods combined with manual methods, as proposed by Santos et al. (2021a). To compose the CL vector, spectral band calculations from Landsat and CL extraction in ArcMap 10.8 software were employed, converting raster (.tiff) features into lines (.shp). In detail, Near Infrared/Shortwave Infrared and Visible wavelength bands from Landsat sensors were utilized to differentiate land and water surfaces along the Coastal Zone (CZ). The infrared wavelength band was preferred because it aids in distinguishing water and land characteristics, where medium infrared energy is absorbed by water (or turbid water) and vegetation, while sand and other coastal features exhibit strong reflectance characteristics in this band (Alesheikh et al., 2007; C. A. G. Santos et al., 2021b).

In this composition, the Normalized Difference Water Index (NDWI) was used because it yielded more satisfactory results than other methods (e.g., visual interpretation of CL) and employs technically enhanced content for LC detection (Mishra et al., 2019; C. A. G. Santos et al., 2021b). The calculation of the Normalized Difference Water Index (NDWI) is

$$\mathrm{NDWeu} = \frac{\mathrm{GREEN\ \text{-}\ NeuRG}}{\mathrm{GREEN+NeuR}}$$

Therefore, NDWI was applied to Landsat images in ArcMap 10.8, following the creation of a TIFF image with the NDWI component for each acquired Landsat image. Finally, it was vectorized to create the outline of CL in shapefile format.

DIGITAL SHORELINE ANALYSIS SYSTEM (DSAS)

All these data were generated using DSAS v5. To perform the calculations, DSAS requires three vector files: 1) the baseline, drawn parallel to the CL either onshore or offshore, i.e., within or outside the mainland, serving as the basis for CL movement calculations; 2) the transects, lines perpendicular to the coast responsible for calculating variations at specific points, segmenting the coastline from the baseline and crossing all available shorelines, automatically generated by DSAS, requiring only the assignment of interval between each transect; and 3) coastline vectors, each assigned to a date within the analyzed temporal range (Honeycutt et al., 2001).

122 transects were drawn perpendicular to the CL with a spacing of 100 meters between them due to the length of the analyzed coastline (~50 km). The settings establish a baseline, located inland relative to the oldest CL (1972), which serves as the starting point for measurements across all generated CL's.

The most commonly used methods for analyzing CL through DSAS are Coastal Net Shore Movement (NSM), End Point Rate (EPR), and Linear Regression Rate (LRR). Therefore, EPR is widely employed on the coast of the United States of America as it uses only two positions of the shoreline to calculate the rates of variation over time (Dolan et al., 1991).

LRR has shown satisfactory results, as demonstrated in the study by Honeycutt et al. (2001), where it was used to calculate long-term erosion rates and predict subsequent positions with a reduction in error of over 70% in New York and 34% in Delaware, including storm-prone coastal areas. Thus, the EPR and LRR analysis components have been highly successful in analyzing CL variation and are consequently the most widely used methods in the scientific field for coastal studies with DSAS (Honeycutt et al., 2001).

NSM measures the distance between the oldest and newest shorelines for each transect. Positive values indicate seaward movement, while negative values indicate landward movement of the CL (Himmelstoss et al., 2018; Kabir et al., 2020).

This article employs the NSM, EPR, and LRR methods, widely accepted in the scientific community (Nascimento, 2012; Mahapatra et al., 2014a; Misra & Balaji, 2015;

Almonacid-Caballer et al., 2016; Kabir et al., 2020; Muskananfola et al., 2020; C. A. G. Santos et al., 2021b), yielding satisfactory results. The rationale for using these methods lies in their ability to accurately calculate CL variation rates and identify medium-term behavioral trends through the coefficient of determination (R^2), where the calculation is:

$$R^2 = 1 - \sqrt{\frac{\sum(y - y')^2}{\sum(y - \bar{y})}}$$

Where,

R^2 is the coefficient of determination;

y is the measured distance from the baseline to a CL data point;

y' is the predicted distance from the baseline based on the equation of the best-fit regression line; and

$\bar{y}$ is the mean of the measured distances of CL from the baseline.

In this sense, R^2 or the Coefficient of Determination, indicates a close relationship between the generated and analyzed data. A value of R^2 close to 1 signifies a satisfactory correlational trend in the data. This suggests a perspective of continuity in the ongoing process (accretion). Conversely, if R^2 is close to 0, it indicates an unsatisfactory correlation among the analyzed data. In such cases, it suggests a perspective of stagnation or discontinuity (erosion) in the current coastal conditioning process (Allan et al., 2003; Maiti & Bhattacharya, 2009; Himmelstoss et al., 2018).

RESULTS

The multi-temporal variability of CL constitutes a scalar-temporal parameter spanning a medium period (1972-2020) in the CZ of the municipality of Soure (~50 km) (Tables 3 and 4). Following the application of DSAS v5, 282 transects were obtained to measure CL changes (Figure 4). Erosion was observed in 160 transects, while accretion was detected in 122 transects (Figure 4; Table 3). Thus, at the mouth of the Pará River estuary, between 1972 and 2020, CL experienced erosion in 57% of transects and accretion in 43%.

The results showed that the maximum erosion distance was -1,247.46 m, while the maximum accretion distance was 1,012.72 m, observed on the southeast (SE) shore of Soure. The findings indicate certain regions with maximum erosion rates, notably the SE coast (-29.41 m/year) (Figure 4; Tables 3 and 4).

The dynamic complexity of the study object needs to be understood in segmented sections. Given that there are dynamic processes acting on the analyzed coastline differently, the following subtopics are necessary to address and focus on the results more directly.

SECTOR I – PARÁ RIVER – SE

Sector I (~27 km) comprises a set of 160 transects (497-656). Statistical modeling quantification reveals a predominant erosive trend. Accordingly, 46 transects (29%) showed accretion tendencies, while 114 transects (71%) exhibited erosive characteristics, with an average coastline retreat rate of -2.1 m/year (Figure 4).

The parameter $R^2 > 0.5$ includes 87 transects, whereas 73 transects with $R < 0.5$ were identified, comprising 54% and 46% for their respective segments. These data indicate a perspective of ongoing processes in the CZ. In this scenario, there is an alternation of dynamic processes; according to R^2 statistics, sedimentation predominates. However, intense erosion is also identified in the quantified data (Table 3).

NSM results indicate an erosive trend of the CL. The average rate from this method is -113.81 m. The highest erosion rate recorded was -1,247.46 m (transect 586), with an average of -193.5 m. Conversely, the advancement of the CL shows a rate of 1,012.72 m (transect 583), with an average rate lower than that of erosion, at 110.07 m (Figures 4 and 5A, Table 4).

The retreat of the CL in this sector is notable, highlighting this trend. Analytically, through the EPR and LRR methods, there is a significant erosion vector in this segment. Quantified data reveal an average variability of -2.38 m/year and -2.13 m/year, respectively, for the two methods. The maximum

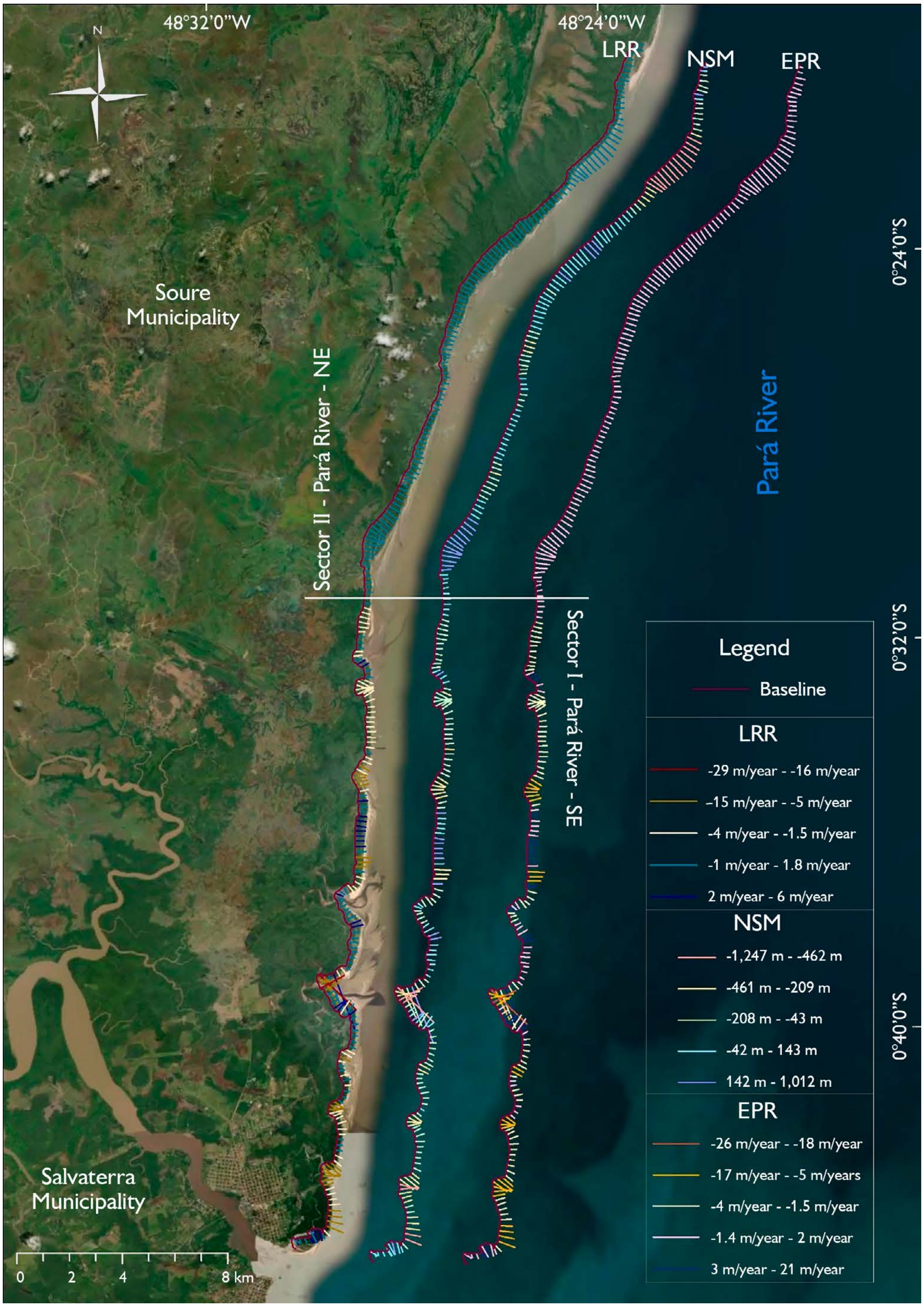

Figure 4. Map of End Point Rate (EPR) and Linear Regression Rate (LRR) at the western edge of the Pará River estuary, municipality of Soure, Marajó Island, Pará, 2020. Source: Author (2024).

erosion retreat is -26.6 m/year (transect 586), averaging -3.53 m/year among negative EPR values. In contrast, LRR exhibits -29.41 m/year (transect 586), averaging -3.94 m/year among erosive values (Figures 4, 5B and 5C, Table 4).

Accretion values the CL show a maximum positive rate of 21.59 m/year (transect 583), with an average of 3.82 m/year, and 5.83 m/year (transect 583), with an average variability of 2.34 m/year, for the underlying methods highlighted (Figure 4).

SECTOR II – PARÁ RIVER – NE

Sector II (~23 km) comprises 122 transects (375-496), where the rates from statistical models reflect a dynamic characterized by stabilization tendencies with erosive trends. It is evident that 76 transects (62%) exhibit stabilization tendencies, while 46 transects (38%) show erosive tendencies. However, erosive rates surpass those of accretion transects, resulting in a coastline variation of 0.2 m/year (Figure 4).

Table 3. Coastline change rates from Zone-I to Zone-II (1972–2020). Source: Author (2024).

Descriptive statistics	Sector I	Sector II	Total
Transect ID	497-656	375-496	375-656
Total number of transects	160	122	282
Coastline length (km)	27	23	50
Total number of transects where erosion was recorded	114	46	160
Total number of transects where accretion was recorded	46	76	122
Total number of transects where statistical uncertainty ($R > 0.5$) was recorded	87	26	113
Total number of transects where statistical uncertainty ($R < 0.5$) was recorded	73	96	169
% of total number of transects where erosion was recorded	71	38	57
% of total number of transects where accretion was recorded	29	62	43
% of total number of transects where statistical uncertainty ($R^2 > 0.5$) was recorded	54	21	40
% of total number of transects where statistical uncertainty ($R^2 < 0.5$) was recorded	46	79	60
Mean coastline change (m/year)	-2.1	0.2	-0.95
Maximum positive coastline change (m/year)	5.8	10.7	10.7
Maximum negative coastline change (m/year)	-29.4	-12.44	-29.4
Average accretion rate (m/year)	2.3	3.2	2.75
Average erosion rate (m/year)	-3.9	-4.8	4.35

Table 4. CL change rates for sectors I and II calculated using NSM (m), EPR, and LRR (m/year) methods between 1972–2020. Source: Author (2024).

Sectors	I				II	
Estatistics	NSM (m)	EPR (m/year)	LRR (m/year)	NSM (m)	EPR (m/year)	LRR (m/year)
Maximum negative rate	-1,247.46	-26.6	-29.41	-635.72	-13.55	-12.44
Maximum positive rate	1,012.72	21.59	5.83	490.81	10.46	10.68
Mean of negative rates	-193.5	-3.53	-3.94	-200.25	-4.3	-4.82
Mean of positive rates	110.07	3.82	2.34	119.75	2.55	3.23
Averages	-113.81	-2.38	-2.13	-51.54	-1.09	0.19

Statistical uncertainties with $R^2 > 0.5$ correspond to 26 transects, whereas 96 transects have $R^2 < 0.5$, representing 21% and 79%, respectively. These uncertainties indicate a trend towards stability or potential erosion over time (Table 3).

Net shoreline movement (NSM) rates show a trend towards stabilization despite sediment input, with an average of -51.54 m considering all negative and positive rates. The maximum erosion/recession rate is -635.72 m (transect 392), with an average rate of -200.25 m. In contrast, the maximum positive rate reaches 490.81 m (transect 486), with an average rate of 119.75 m (Figures 4 and 6A, Table 4).

EPR and LRR methods indicate rates skewed towards erosive processes. Specifically, average variabilities are -1.09 m/year and 0.19 m/year, respectively. The maximum erosive rate reaches -13.55 m/year (transect 392) with an average of -4.30 m/year for EPR, and -12.44 m/year (transect 392) with an average of -4.82 m/year for LRR. Meanwhile, the maximum positive rates are 10.46 m/year (transect 486) with an average of 2.55 m/year, and 10.68 m/year (transect 486) with an average variability of 3.23 m/year, as observed through the methods applied (Figures 4, 6B and 6C, Table 4).

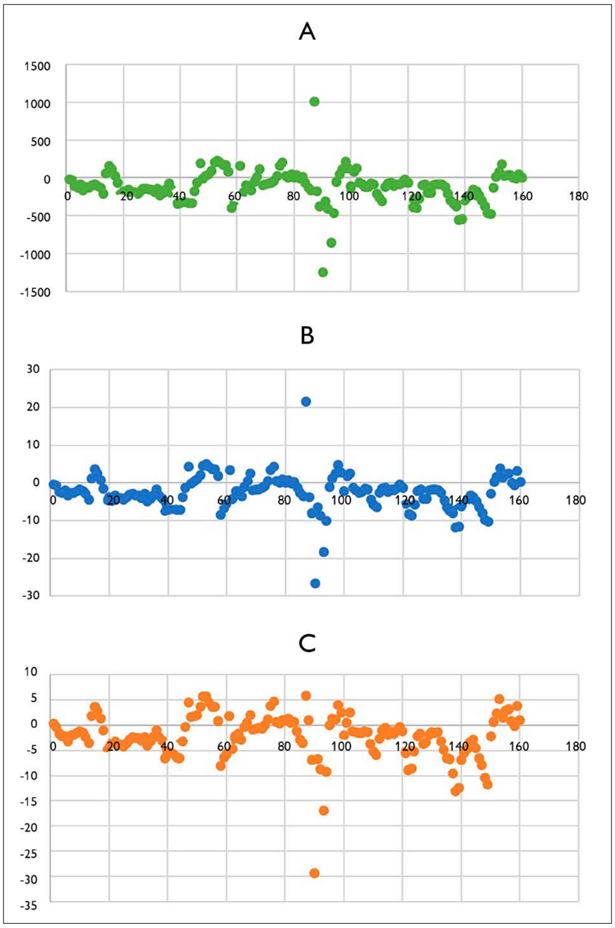

Figure 5. Net Shoreline Movement (NSM-A), End Point Rate (EPR-B), and Linear Regression Rate (LRR-C) in Sector I of Soure municipality, Marajó Island, Pará. Source: Author (2024).

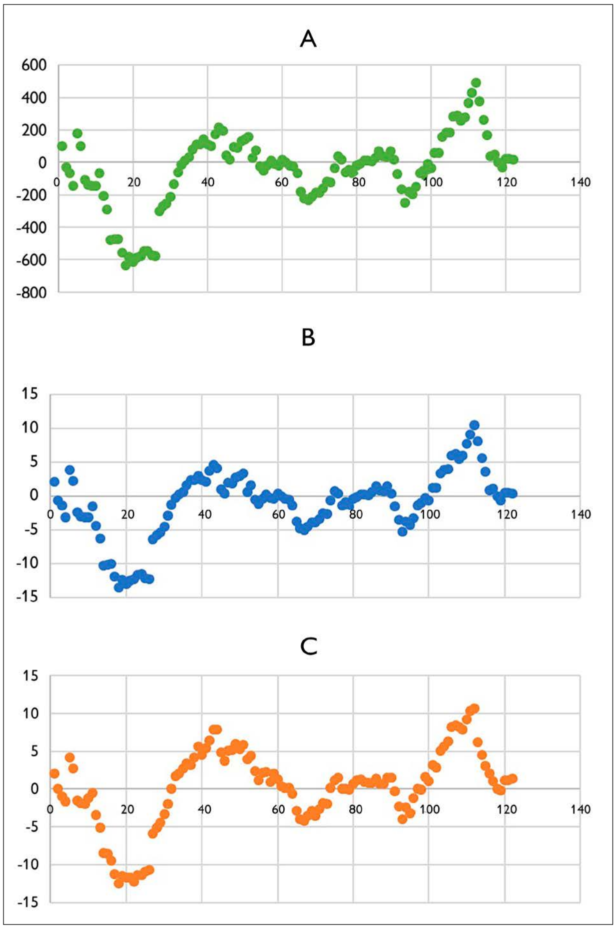

Figure 6. Net Shoreline Movement (NSM-A), End Point Rate (EPR-B), and Linear Regression Rate (LRR-C) in Sector II of Soure municipality, Marajó Island, Pará. Source: Author (2024).

DISCUSSION

The scenario of sectors I and II, where both are influenced by the dynamics of the waters from the Pará River estuary, characterized by a predominance of semidiurnal macrotides with secondary importance of diurnal oscillations (both astronomically driven), is generated due to the nonlinear interaction among the main harmonic constituents, especially the M4 (Prestes et al., 2020). According to the same authors, tidal currents (2 m^3s^{-1}) associated with river discharge (20,946 m^3s^{-1}) favor erosive effects, as the energy distribution associated with CL on the left bank of the Pará River acts as a remover of sediment particles, favoring accretion on the right bank of this river. On the eastern bank, the structure corresponding to the Barreiras sediments is represented by sandstones and claystones followed by Post-Barreiras sediments (Rossetti et al., 2008a, 2008b), which identifies the accretive content and type of deposits in this spatial scenario.

In sector II, the average LC variation is 0.2 m/year, and the average among all NSM rates, negative and positive, corresponds to -51.54 m. It exhibits a maximum erosion rate of -635.72 ± 200.25 m, whereas the maximum positive rate is 490.81 m ± 119.75 m. The EPR and LRR average -1.09 m/year and 0.19 m/year, respectively, indicating a trend towards erosion.

Sector I, on the other hand, is typically erosive with an average LC retreat rate of -2.1 m/year. The NSM averages -113.81 m, with a maximum erosive rate of -1,247.46 m ± 193.5 m, and a positive scenario of 1,012.72 m ± 110.07 m. The EPR averages -2.38 m/year, and LRR averages -2.13 m/year.

The western bank exhibits a tendency towards erosion, considering the erosive effects in the South Channel of the Amazon River, where strong tidal currents are linked to tides (maximum of 4.2 m at spring tide, with a variation of 0.5-1.2 m between neap tide or quadrature) (Rosário, 2016). This is due to the Pará River estuary experiencing intense riverine input combined with the co-oscillation of astronomical tides, resulting in a distinct hydrodynamic pattern and complex mixing process. Sediments on this bank consist mainly of medium to fine sand (Corrêa, 2006). Another factor to consider is that this bank is more exposed to winds entering the South Channel at speeds ranging from 2 m/s to 7 m/s (INMET, n.d.), which contributes to increased erosion.

The accretion of the CL in this geographical area is related to the distribution pattern of suspended material, where it reaches a maximum of 0.385 $m^{-1}s^{-1}$ and a minimum of 0.112 $m^{-1}s^{-1}$ without current inversion (Carneiro et al., 2020) (Figure 7). Thus, the data obtained by Carneiro et al. (2020) estimated that the export of suspended solids (TSSL) reached a maximum value of 0.9229 kg $m^{-1}s^{-1}$ in the dry season and 0.6650 kg $m^{-1}s^{-1}$ in the rainy period. This condition is also influenced by the Amazon River plume, which deposits sediments in the vicinity of the Pará River estuary margins. Furthermore, the penetrating tides are important for regulating the dynamics of suspended solids in estuaries and, particularly, in the Pará River estuary, where the river discharge is 20,946 m^3s^{-1} (Prestes et al., 2020), imparting to this region a significant kinetic energy perpendicular to the CL, where the net input of the continental regime reacts and integrates directly with the barotropic flow generated by tidal currents.

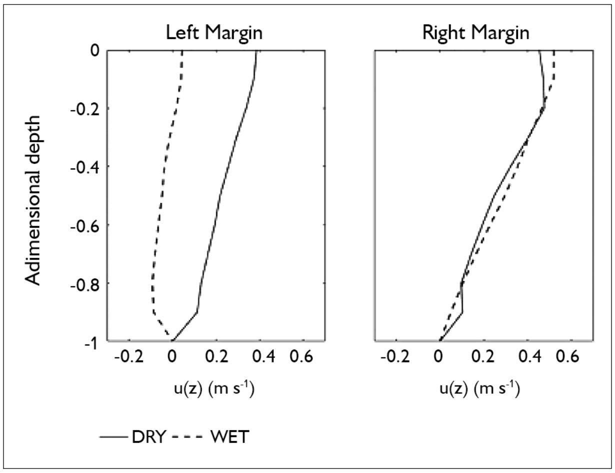

Figure 7. Vertical residual profiles of the u-velocity component for the dry season (solid line) and rainy season (dashed line) on both banks of the Pará River estuary. Source: Prestes et al. (2020).

Thus, some researchers reached similar results (Baig et al., 2020; Benkhattab et al., 2020; Galvez et al., 2020; Kabir et al., 2020; Mishra et al., 2020; Muskananfola et al., 2020; Warnasuriya et al., 2020; C. A. G. Santos et al., 2021b). Santos et al. (2021), on the beaches of Bessa and Intermares Campina, using the EPR method, reported values of -1.20 m/year and 0.94 m/year for the maximum and minimum rates, respectively. Using the LRR method, the minimum and maximum rates of CL change were -0.90 m/year and 1.22 m/year, which indicates a process similar to the dynamics occurring in sector II.

In contrast, for Ponta do Seixas beach, these authors reported rates for the EPR (-0.39 m/year) were higher than the LRR values (-0.32 m/year), indicating an amplitude of erosive character, similar to sector I, located southeast of Soure.

The rainy and dry seasons influenced by the ITCZ (Intertropical Convergence Zone) and the El Niño (EN) and La Niña phenomena (Figure 8) are also relevant to the estuary's flow. In an average analysis period, possible changes related to extreme events are considered in addition to seasonal changes. All factors that influence river discharge, currents, and tides, whether over a long or short period, are relevant to coastal changes.

The CL's of the beaches that are the subjects of this manuscript were influenced by these factors. In Figure 8, it is possible to observe the temporal alternations of extreme events related to EN and LN. In the context of EN, it influenced sediment input, especially during its 'very strong' characteristic phases occurring in 1990-1993, 1997-1998, and 2015-2016. This extreme phenomenon affects the climate of the Amazon, characterized by a significant reduction in rainfall, which decreases the flow of the Pará River and, consequently, fluvial erosion. Thus, opposite extreme conditions can occur, focusing on the transition to LN. This extreme, unlike EN, causes a substantial increase in sediment input due to the association with the ITCZ and the displacement of the Walker cell (INMET, 2021). These extreme events, combined with anthropogenic and hydrographic/oceanographic factors, were fundamental in the erosive reworking and accretion in the study area, especially during the analysis period of the CL's.

FINAL CONSIDERATIONS

The methodology employed, using semi-automatic methods derived from the spatial analysis of orbital images acquired by remote sensors (Landsat) and utilizing DSAS v5, has enabled significant scientific advancements for the Amazon region and holds global importance. Throughout the manuscript, geoprocessing tools and techniques are used that can be replicated in other CZ, systematically contributing technical, theoretical, and methodological support for policy makers in management.

The challenges of implementing this set of techniques are numerous. However, the acquisition of a more accurate temporal and spatial dataset, with less cloud cover, stands out as nearly unfeasible due to the

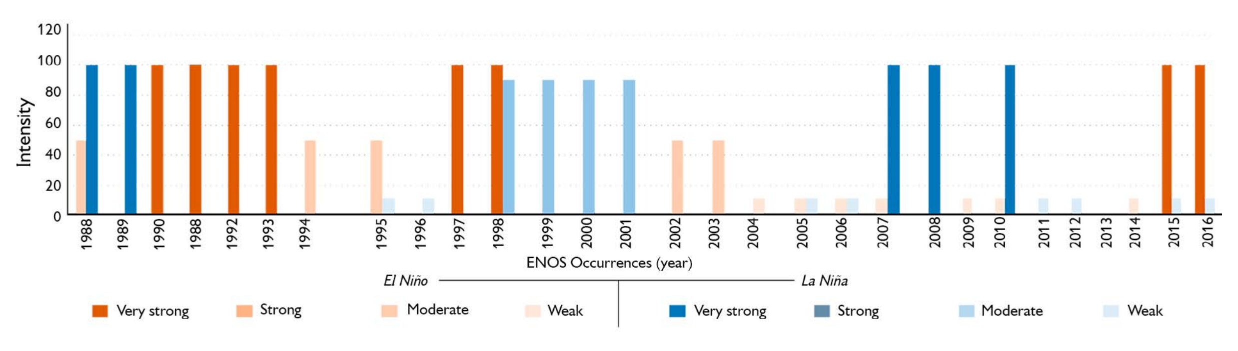

Figure 8. Historical occurrences of El Niño and La Niña. Source: CPTEC (2019).

study area being located in the ITCZ. An alternative to address this issue is the use of active sensors (synthetic aperture radar) and/or photogrammetry from unmanned aerial vehicles (UAVs), integrating them with the data available and utilized in this study and others under the perspective of this manuscript.

The results made it possible to identify that each area of the sector is marked by different dynamics according to its exposure to coastal factors. Thus, the results achieved are relevant, as the analysis can be used by public administrators considering that the sectors of the eastern margin of Soure municipality do not exhibit uniform dynamics throughout their extent.

Despite the significant progress made through this work, more in-depth analyses of the processes controlling the retreat and accretion of the CL in this geographical area are still necessary. The information obtained in this study summarizes only some parameters (statistical rates and CL mobility) of the dynamics, which are suitable for case studies (in situ). A greater acquisition of recent oceanographic and geological data, as well as a comprehensive set of orbital data, is essential because this region is still underrepresented in integrated studies regarding these scientific areas. This will allow for the evaluation of probable predictive scenarios for the evolution of the CL.

Finally, the analysis conducted in this region, integrated with the processes affecting the CL of Marajó Island (where the study area is located), provides a fragmentary understanding of a more complex dynamic. However, it can contribute as a basis for future research on coastal dynamics in other regions, adding informational mechanisms that serve as a predictive model for environmental changes on a large temporal and spatial scale using remote sensing and geoprocessing techniques.

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AUTHORS' CONTRIBUTION

R. A. A. Menezes contributed to conceptualization, data curation, investigation, methodology and writing (original draft, review and editing); D. K. M. Guimarães contributed to writing (review and editing); and M. El Robrini contributed to supervision and writing (review and editing).

Definition of the number of instars, description of the second instar and redescription of the last instar and pupa of *Pantophthalmus kerteszianus* (Enderlein, 1914) (Diptera: Pantophthalmidae)

Definição do número de ínstares, descrição do segundo ínstar e redescrição do último ínstar e pupa de *Pantophthalmus kerteszianus* (Enderlein, 1914) (Diptera: Pantophthalmidae)

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Abstract: The total number of larval instars of *Pantophthalmus kerteszianus* was determined through morphological and biometric studies. The second larval instar of *P. kerteszianus* is described, the last larval instar in detail and the pupa are redescribed. Larvae were collected from *paricá* trees (*Schizolobium parahyba* var. *amazonicum*) in the Paragominas county, Pará, Brazil. Upon collection, larvae and pupae were killed in boiling water, preserved in 70% alcohol, identified, measured and morphologically studied. The total number of larval instars was determined by techniques based on Dyar's rule (1890), supported by measurements of the several characters: body length, maximum body width, maximum diameter of the prothoracic spiracle, maximum diameter of the anal spiracle, distance from the margin of the prothoracic spiracle to the lower bristle, distance from the margin of the prothoracic spiracle to the prothoracic margin, width of the median band of the prothorax and distance between the anal spiracles. Through these measurements it was possible to establish that *P. kerteszianus* has seven larval instars; this species is, up to now, the only one having the number of its larval instars determined. Photographs and drawings of the complete larvae and pupae, as well as of the most important structures discussed in the descriptions, are provided.

Keywords: *Pantophthalmus kerteszianus*. Timber flies. Immatures. Instar number.

Resumo: O número total de ínstares larvais de *Pantophthalmus kerteszianus* foi determinado através de estudos morfológicos e biométricos. O segundo ínstar larval de *P. kerteszianus* é descrito, o último ínstar larval e a pupa são redescritos. As larvas foram coletadas em árvores de paricá (*Schizolobium parahyba* var. *amazonicum*) no município de Paragominas, Pará, Brasil. Depois de coletadas, as larvas e pupas foram mortas em água fervente, conservadas em álcool 70%, identificadas, medidas e estudadas morfologicamente. O número total de ínstares larvais foi determinado por técnicas baseadas na regra de Dyar (1890), apoiadas em medidas dos seguintes caracteres: comprimento do corpo, largura máxima do corpo, diâmetro máximo do espiráculo protorácico, diâmetro máximo do espiráculo anal, distância da margem do espiráculo protorácico até a cerda inferior, distância da margem do espiráculo protorácico até a margem protorácica, largura da faixa mediana do protórax e distância entre os espiráculos anais. Através dessas medidas foi possível estabelecer que *P. kerteszianus* possui sete ínstares larvais; esta espécie é, até o momento, a única que tem o número de ínstares larvais determinado. São fornecidas fotografias e desenhos das larvas e pupas, bem como das estruturas mais importantes tratadas nas descrições.

Palavras-chave: *Pantophthalmus kerteszianus*. Mosca da madeira. Imaturos. Número de ínstares.

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INTRODUCTION

Pantophthalmidae is a small family of the order Diptera, suborder Brachycera, exclusive to Neotropics and geographically distributed primarily in South and Central America. A few species occur in Mexico and the Antilles (Carrera & D'Andretta, 1957; Val, 1976).

The larvae are wood-borers, that attack various species of both native and introduced trees (list in Papavero, 2009a). Lunz et al. (2010) recorded larvae *Pantophthalmus* spp. in paricá (*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby) in the state of Pará, Brazil. Rapp (2011) describes the immatures of *P. bellardii* (Bigot) and *P. kerteszianus* (Enderlein).

Twenty species of Pantophthalmidae are known, belonging to two genera: *Opetiops* Enderlein 1921, with only one species, *O. alienus* (Hermann, 1916), and Pantophthalmus Thunberg 1819, with nineteen species (Val, 1976). Nine species of Pantophthalmidae occur in the Brazilian Amazon, all in the genus *Pantophthalmus* (Papavero, 2009b). Of the twenty existing species of Pantophthalmidae, only five have described larvae, so it is important to understand the immature stages of the other species.

This work describes the second instar larvae for the first time and redescribes the last instar larva and pupa of *Pantophthalmus kerteszianus* (Enderlein, 1914). It also determines, for the first time, the number of larval instars of a species of the family Pantophthalmidae, based on morphological and biometric characteristics. Variations in the structures measured in the instars and differences between the larvae and pupae of *P. kerteszianus* compared to *P. tabaninus* (Greene and Urich, 1931) and *P. palniventris* (Wiedemann, 1821) are presented.

The work presents photos, drawings and a diagram of the body division of *P. kerteszianus* that facilitate the visualization of the structures described.

MATERIAL AND METHODS

Pantophthalmus. kerteszianus larvae were collected in trunks of paricá in August 2011 at the Paragominas, Pará, Brazil, in coordinates 03° 38' 976" S, 48° 50' 449" W. The collected larvae and pupae were killed in boiling water, preserved in 70% alcohol for subsequent identification, measurement, and morphological analysis to determine larval instars and describe the immature stages.

The following characters were analyzed and measured: body length, greatest body width, greatest diameter of the prothoracic spiracle, greatest diameter of the abdominal spiracle, distance from the margin of the prothoracic spiracle to the upper bristle, distance the margin of the prothoracic spiracle to the lower bristle, distance from the margin of the prothoracic spiracle to the margin of the prothorax, width of the median band of the prothorax and distance between the anal spiracles (Table 1 and Figures 1A-1H). These structures were selected because less subject to variations arising from feeding, for being are more robust due to sclerotization and contain fewer membranous areas. Only a single second instar larva was collected, and for that reason it was not included in the calculation. No first instar larvae were collected.

Mathematical average values and standard deviations were calculated, allowing the construction of graphs with the analyzed structures. By examining the frequency intervals, the stages of *P. kerteszianus* was defined. These procedures were based on Dyar's rule (Dyar, 1890) and the methodology described by Parra and Haddad (1989).

The identification of the larvae was based on the adults obtained in laboratory from the pupae collected in the *paricá* trunks. They were identified with assistance of Val's revision (Val, 1976). Morphological terminology was based on the works of Greene and Urich (1931), Peterson (1960), Teskey (1981) and Stehr (1987).

The specimens are deposited in the entomological collection of the *Museu Paraense Emílio Goeldi*, Belém, Pará, Brazil.

Drawings of the immatures and measurements were made with a Zeiss SV11 stereoscopic microscope with camera lucida and a micrometric lens. Digital photographs of larvae and pupae of *P. kerteszianus* were captured with a Leica MZ16 camera adapted to a Leica DFC 420

Table 1. Average and standard deviation of measurements of the body structures of larval instars II-VII of *Pantophthalmus kerteszianus*. Legend: * = measurements of the structures of the second larval instar were taken from a single specimen collected. The lower bristle of the prothoracic spiracle and the bands of the prothorax do not exist in the second instar; the anal spiracles were not measured, in order not to damage the specimen.

Measured structures (mm)	Larval instars					
	II*	III (n = 22)	IV (n = 32)	V (n = 61)	VI (n = 22)	VII (n = 7)
Body length	12	12.72 ± 2.62	22.90 ± 1.69	29.59 ± 2.47	41.37 ± 3.16	55.33 ± 3.16
Body width	1.21	3.87 ± 0.53	6.73 ± 0.46	8.05 ± 0.28	9.02 ± 0.32	10.53 ± 0.44
Diameter of the prothoracic spiracle	0.16	0.35 ± 0.03	0.51 ± 0.04	0.58 ± 0.01	0.73 ± 0.08	0.92 ± 0.05
Distance from the prothoracic spiracle to the upper bristle	0.28	0.13 ± 0.02	0.24 ± 0.02	0.32 ± 0.02	0.40 ± 0.03	0.55 ± 0.03
Distance from the prothoracic spiracle to the lower bristle	-	0.15 ± 0.03	0.22 ± 0.01	0.29 ± 0.03	0.38 ± 0.01	0.46 ± 0.02
Distance from the spiracle to the prothoracic margin	0.34	0.49 ± 0.10	0.85 ± 0.06	1.15 ± 0.12	1.58 ± 0.07	1.8 ± 0.07
Width of the median band of the prothorax	-	0.64 ± 0.11	1.24 ± 0.12	1.58 ± 0.07	1.84 ± 0.11	2.39 ± 0.02
Diameter of the anal spiracle	-	0.41 ± 0.03	0.63 ± 0.05	0.80 ± 0.05	1.00 ± 0.08	1.31 ± 0.06
Distance between the anal spiracles	-	0.29 ± 0.06	0.64 ± 0.07	0.81 ± 0.03	0.95 ± 0.05	1.47 ± 0.25

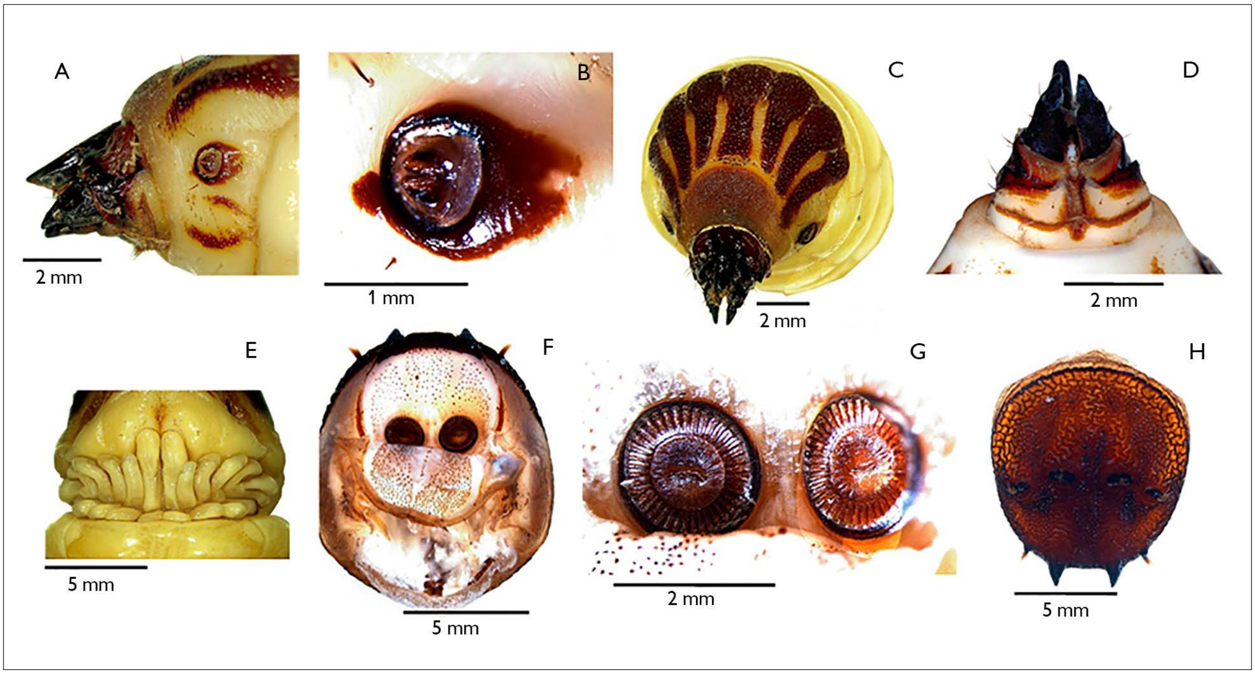

Figure 1. *Pantophthalmus kerteszianus*, larval body structures: A) head and prothorax, lateral view; B) prothoracic spiracle (in figures A and B may be seen the spiracle); C) head and prothorax, frontal view; D) head, ventral view; E) abdominal segments 7 and 8, ventral view; F) 8th abdominal segment, posterior view, with detail of opening of the anal spiracles; G) anal spiracles; H) sclerotized plate of 8th abdominal segment, posterior view. Images: I. S. Gorayeb (2014).

stereoscopic microscope, operated using Leica IM50 software, version 5.03.0061, release 247, and the photo editor software Auto MontagePro, version 5.

RESULTS AND DISCUSSION

DETERMINATION OF THE NUMBER OF LARVAL INSTARS OF *P. KERTESZIANUS*

The number of larval instars was determined through biometric studies and confirmed by the several measured structures, based on Dyar's rule (Dyar, 1890). Averages and standard deviations of the nine analyzed structures are shown in Table 1. Measurements were taken from a total of 144 larvae of various sizes. Data of each measured structure were applied in frequency intervals where peaks and gaps allowed the determination of the larval instars. Averages and standard deviations of the specimens within each interval of frequency were then calculated; and these data were used to generate graphs displaying the trend line of tendency, the function and the coefficient of determination (R^2) (Table 1, Figures 2A-2I).

The calculi made it possible to determine that *P. kerteszianus* shows seven larval instars, as proven by the measurements of all the structures chosen for analysis, some of them presenting less variation.

VARIATION OF THE STRUCTURES MEASURED IN THE INSTARS

In general, the measured structures exhibited relatively little variation, as evinced their standard deviations; indicating that all could be effectively used to distinguish the larval instars of *P. kerteszianus* (Table 1 and Figures 2A-2I). Nonetheless, as expected, structures with membranous connections between segments and those that are less sclerotized displayed greater variability. Specifically, body length and width showed the highest variation, whereas the distance from the margin of the prothoracic spiracle to the lower bristle and the width of the median band of the prothorax demonstrated relatively lower variation.

No significant difference was observed among the analyzed characters between instars III and VII – except for body length. The second instar exhibited many differences as compared to the remaining instars which is why it is described in detail. No specimens from the first instar were available.

The descriptions below are presented in the following order: last (seventh) instar, second instar larva and pupa.

REDESCRIPTION OF THE LAST LARVAL (SEVENTH) INSTAR OF *PANTOPHTHALMUS KERTESZIANUS*

The last (seventh) larval instar of *P. kerteszianus* is cylindrical, with an average body length of 55,33mm and a width of 10,53 mm (Table 1; Figures 3A-3C).

Head: Hemicephalic, strongly sclerotized, and black, with a transition to brown toward the dorsolateral proximal margin; the ventral and lower lateral areas are white, bearing brown spots near the mandible and on the central area. The ventral surface exhibits a brown line fringed with densely set bristles. The frontoclypeus and labrum form a strongly chitinized, dorsal snout located in a sulcus near the dorsal region, which bears pale bristles; centrally, a larger, pale bristle is present in a depression, and on the proximal third of the margin of the frontoclypeal suture, another pale bristle is observed. The integument of the gena is yellowish-brown, with an irregular surface featuring raised areas, depressions, and black transverse grooves resembling cracks; additionally, an upper bristle is located near the margin of the frontoclypeal suture and three bristles on the proximal ventral area. The mandible is strongly sclerotized and black. At the site where the antenna is implanted, a plaque is present, located anteriorly to the gena, between the snout and the base of the mandible. The antenna is centered on a white area and contains a small, grayish cone; the mandible is sclerotized, black, with a dorsal tooth; below this tooth, within a sulcus, there is a large bristle (the largest one in the head). Along the internal margin of the mandible is the prostheca, with bears a tuft of yellowish bristles. More distally the maxilla is situated, with the maxillary palpus located on the external margin; the palpus, similarly to

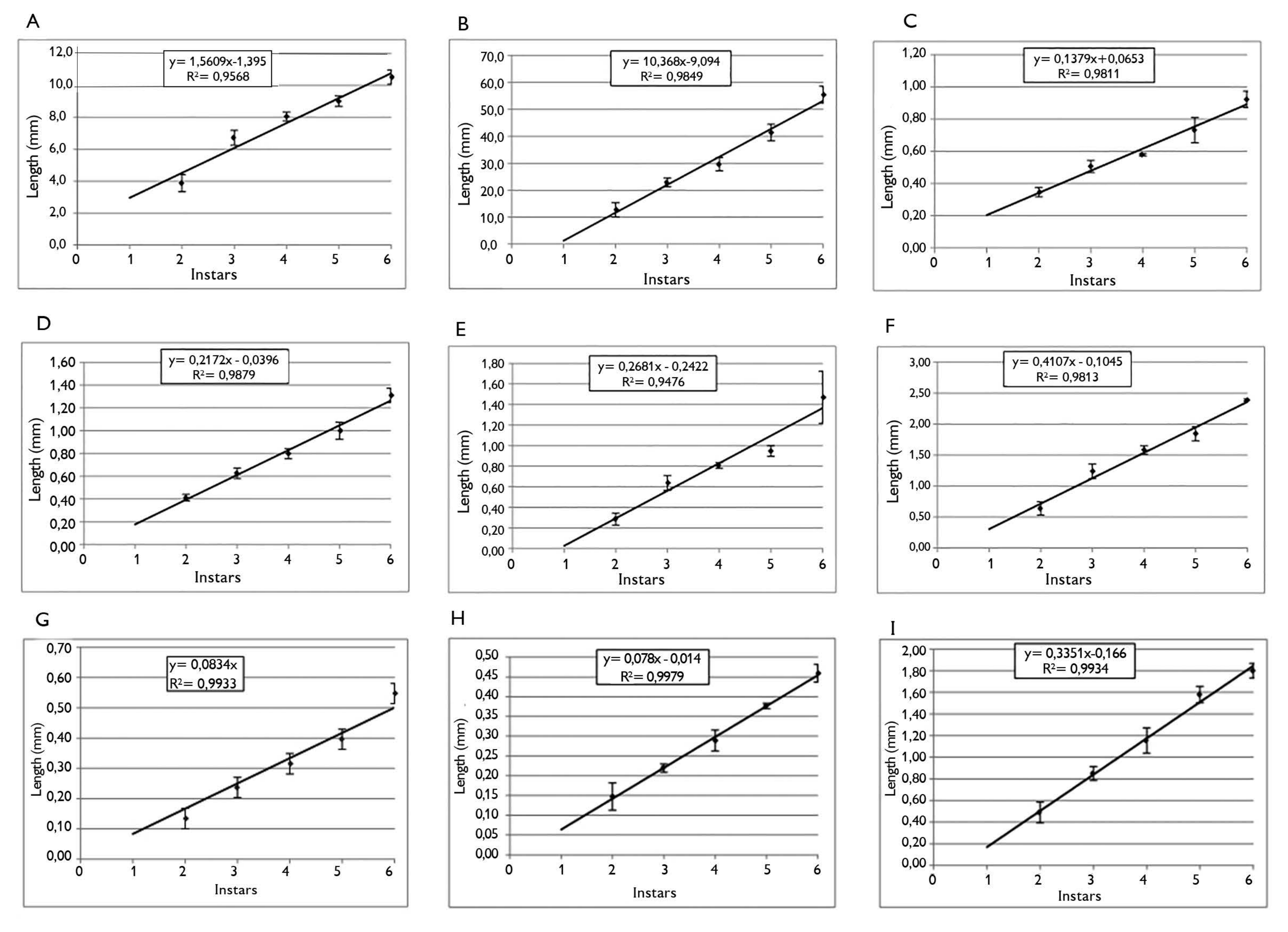

Figure 2. Averages and standard deviations of the measured structures of *Pantophthalmus tabaninus* larval instars, with lines of tendency, functions (y) and coefficients of determination (R_2): A) body width; B) body length; C) diameter of prothoracic spiracle; D) diameter of anal spiracle; E) distance between abdominal spiracles; F) width of median band of prothorax; G) distance from the prothoracic spiracle to the upper bristle; H) distance from the prothoracic spiracle to the lower bristle; I) distance from the prothoracic spiracle to the margin of the prothorax. Graphics: L. E. C. Cardoso (2014).

the antenna, the palpus is inserted on a white area with a grey cone; the ventral integument of the mandible presents about 15 transversal grooves (Figures 1A and 1D).

Thorax: The prothorax, along with the last abdominal segment, exhibits the greatest concentration of dark and sclerotized structures, in addition to the spiracle and setae. Its dorsolateral area displays brown spots arranged in seven longitudinal bands, with a longer central one and six lateral ones (three on each side); among the bands, on the intervening white area, there is a pair of bristles – one basal, the other distal (Figure 1C); the integument of the brown bands is composed by small, circular, inflated, dark-brown areas and clearer oval depressions. At the distal margin of the prothorax there is a spot that nearly encircles the entire segment. This spot it is formed by small, circular, inflated similar to those observed in the longitudinal bands; however, it decreases in size in the lateral areas, reducing to micropilosity; while the ventral area remains bare. The prothoracic spiracle with an internal diameter of 0.92 mm in average (Table 1), is laterally positioned and features two ovoid internal structures parallelly placed anteroposteriorly, situated from the middle towards the proximal margin of the spiracle and are light brown in the center and darker in the margin; the remaining white central area opens towards the body; the borders are well sclerotized and black; around the spiracle there is an irregular brown spot; two lateral, brown spots below the spiracle and a bristle situated above the spiracle (at a distance of 0.55 mm, in average) and a bristle below the spiracle (at a distance of 0.46 mm, in average) are present (Table 1). Two more bristles are present further down on the lateroventral area of the prothorax (Figures 1A and 1B).

Lateral stripes of the body: The larval body is laterally marked, from the mesothorax to the 6th abdominal segment, by two distinct stripes (Figures 3A and 4). These consist of punctiform tegumentary spots or small traits within tegumentary sulci. The median area between the stripes is slightly more inflated and whitish. The pattern of the spots forming the stripes is similar in the meso- and metathorax. On the upper abdominal segments, the stripe is formed by a single continuous sequence of points, whereas the lower stripe is broken up into two sequences; on the abdominal segments 2-6, the rear sequences of the anterior stripe curves upward from the preceding section. In contrast, the 7th segment does not show well defined stripes as observed in the previous segments, only two sinuous lines formed by tegumentary spots. Additionally, the inferior surface of the mesothorax, of the methatorax and of the abdominal segments 1-7, below the lower stripe, is slightly more yellowish than the surrounding areas.

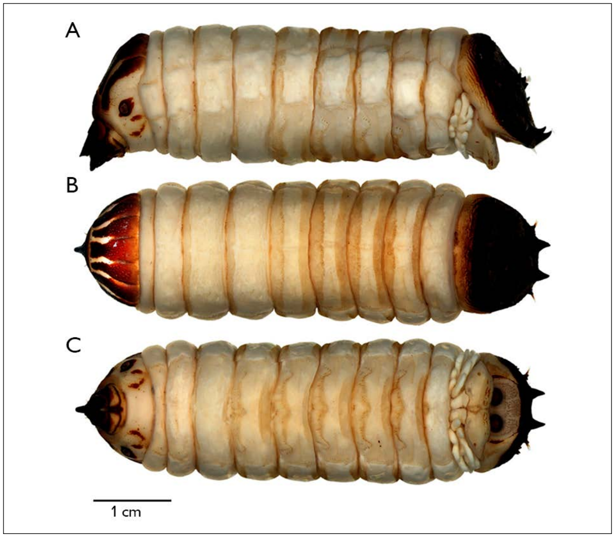

Figure 3. Larva of *Pantophthalmus kerteszianus*: A) lateral view; B) dorsal view; C) ventral view. Images: I. S. Gorayeb (2014).

Mesothorax: White. The segment is white and represents the narrowest portion of the body. Its anterior margin is irregular, with a more pronounced invagination in the lateral regions. Rows of small, faint tegumentary spots are present on both the posterodorsal and ventral regions, and two transversal lateral sulci bear small spots that form l lateral stripes. Bristles: On the dorsal area above the transversal stripe, near the anterior margin, three bristles are arranged in a row. In the median area, between the stripes, four bristles are present, near the margin. Below the inferior transversal stripe five bristles are found, two of these have fused bases and inserted medially and anteroposteriorly; two additional bristles are positioned more anteriorly, also in an anteroposterior

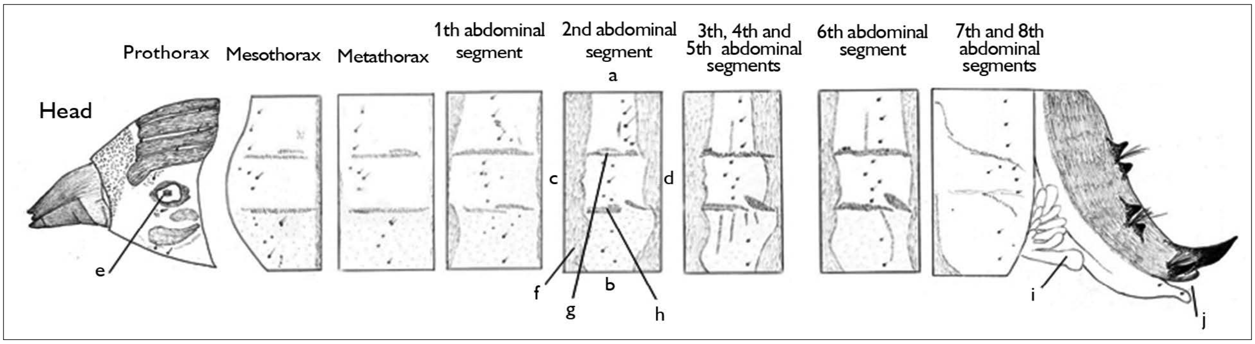

Figure 4. Schematic drawing of the segments of the *Pantophthalmus kerteszianus* larva. Legends: a = dorsal margin; b = ventral margin; c = anterior margin; d = posterior margin; e = prothoracic spiracle; f = tegumentary spots; g = upper lateral stripe of body; h = lower lateral stripe of body; i = branchiae; j = opening of anal spiracles. Illustration: I. S. Gorayeb (2014).

orientation; and a fifth bristle is located ventrally, inserted anteriorly. Tegumentary spots: A narrow stripe of spots is present along the posterior margin (Figures 3 and 4).

Metathorax: The structure is similar to that of the mesothorax but wider, with regular margins; and the ventral bristles are positioned anteriorly. Bristles: They display the same arrangement as those of the mesothorax, except for the lower ventral bristle, which is inserted medially in an anteroposterior direction. Tegumentary spots: A weak, narrow stripe is present along the posterior margin (Figures 3 and 4).

Abdomen: Segments 1-7, display a longitudinal whitish line along the dorsal and lateral surfaces, with the lateral line being slightly more pronounced. On the ventral side, there are patterned, stained areas defined by series of small spots in the anterior and posterior regions of each segment, which are less distinct in the first segment (Figures 3 and 4).

First abdominal segment: In the upper area above the lateral stripe, there are six small circular dark spots. Some of these spots bear bristles arranged dorsoventrally in the central area in the anteroposterior orientation. Two of these points nearest the upper transverse stripe are aligned parallel in the anteroposterior direction. In the median area between the transverse stripes, there are six points, some have bristles arranged in a dorsoventral queue, with three points aligned parallel in a anteroposterior direction. In the ventral area below the transverse stripe, seven points are observed, some stronger and with bristles. Tegumentary spots: The spots along the anterior margin are elongated in both the dorsal and ventral areas, while those located between the transverse strips and along the entire posterior edge are very narrow (Figures 3 and 4).

Second abdominal segment. Bristles: In the upper area above the transverse strip five bristles are arranged in a dorsoventral row, with their orientation positioned posteriorly in an anteroposterior direction. In the median area between the transverse strips, five bristles are arranged medially in the anteroposterior direction, among these, two in the upper part and two in the lower part are almost parallel in an anteroposterior orientation. In the lower area below the transverse strip, there are three bristles. Tegumentary spots: The anterior and posterior margins exhibit wide bands of tegumentary spots (Figures 3 and 4).

Third, fourth and fifth abdominal segments: The number and position of the bristles and tegumentary spots are equal in abdominal segments 3, 4 and 5. Bristles: In the upper area above the transversal stripe, three bristles are arranged vertically, positioned relatively far from the center to the posterior margin. In the median area between the transverse stripes, five bristles are aligned centrally, two of which are oriented parallel to the anteroposterior axis. In the lower area, below the transversal strip, two bristles are arranged vertically, situated slightly off-center toward the posterior margin, arranged in a distinctive pattern. Tegumentary spots: The spots along the anterior and posterior margins are broad. Above the transverse stripes, there is a narrow median line running in a dorsoventral direction toward the upper stripe. In the ventral area below the lower transverse stripe, four narrow lines, also oriented dorsoventrally, are present, with the anterior line being longer than the others (Figures 3 and 4).

Sixth abdominal segment. Bristles: The number and arrangement of bristles in the sixth abdominal segment are the same as those in segments 3 to 5. Tegumentary spots: The tegumentary spot along the posterior margin is narrower than that in segments 3–5 but wider than in segments 1–2. In the upper area, a narrow line—similar to the one in segments 3–5—is present, whereas in the lower area there is a single, longer curved line extending nearly to ventral bristle (Figures 3 and 4).

Seventh abdominal segment. Bristles: in the upper area, three bristles, are arranged in a row, similar to those in abdominal segments 3-6. In the median area between the transverse stripes, five bristles are present, arranged in a pattern that differs from the other segments. In the lower area, two bristles are arranged in a row, as in abdominal segments 3-6. Tegumentary spots: the upper and lower sidebands of the spots are absent, there are two sinuous rows of spots—one in the upper area and another in the median ventral area. The first row starts at the center and extends toward the ventral side, with no additional markings beyond this (Figures 3 and 4).

Respiratory organ: In the ventral median area between segments 7 and 8 there is a milky-white structure with 22 lobes (11 on each side) that are shaped like slightly clavate fingers. This structure functions as a respiratory organ, likely serving as gills that allow the larvae to breathe when the galleries in the tree trunk are filled with water or sap (Figures 1E, 3A, 3C and 4).

Eighth abdominal segment: The morphology of the eighth abdominal segment is quite different from other parts of the body. The fourth former is milky white, similar to the preceding segments. Subsequent three-quarters are dark brown and black, sclerotized with wrinkled tegument, depressions and protrusions. In the median area features two pairs of strong spines black sclerotized; among the thorns of each pair there is a tuft of setae on the ventral region of this plate sclerotized; there is a pair of black thorns sclerotized, a smaller side, with a tuft of bristles at its base medial and another three to four times larger (Figure 1H). In the posterior ventral region there is a cavity where the annals respiratory spiracles are. These spiracles are positioned on the dorsal surface of this cavity (Figures 1F, 1G, 3A and 3C), the distal edge is more sclerotized distally and brown, basally enlarged. In basal edge there is tegumentary spot. At the distal edge of the spiracles, which are separated by bare areas, there are three groups of dark structures that seem sclerotized thorns. The ventral 'language' of the anal opening has edges with weak tegumentary spots, there are three points of bristles on each side of the edge. The internal groove is milky white and has two groups of sclerotized dots that seem spikes separated by a median bare area containing a median line of points. At the distal edge of each dark spot, there is a large bristle. In the outer distal edge of the 'language' there are four stages and one more at the proximal edge. Moreover, tegumentary spots appear on the outer distal edge of the midline and another circular spot with a central point in the lateral median area.

Anal respiratory spiracles: The anal respiratory spiracles have an average internal diameter of 1.31 mm and are separated by an average distance of 1.47 mm (Table 1). Each spiracle features a darker gray outer ring and an inner ring with several radial grooves. Within this inner ring is a darker gray circular area that displays an irregular, linear groove oriented laterally (Figures 1F and 1G).

DESCRIPTION OF THE SECOND LARVAL INSTAR OF *PANTOPHTHALMUS KERTESZIANUS*

Cylindrical; length 12 mm; width 1.20 mm. The head, prothorax and the 8th abdominal segment are more heavily sclerotized than the remaining parts, appearing white, with brown spots formed by tegumentary rugosities. A light milky-white lateral stripe, slightly more inflated than the others on other parts of the body, may be seen from the mesothorax up to the 7th abdominal segment. The head is sclerotized, dark grey. Prothorax sclerotized, brown, covered by rugosities; its spiracle placed on a sclerotized, smooth, dark-brown area (Figures 5 e 6).

Head: Hemicephalic, sclerotized, and uniformly gray, with dark brown coloration along the proximal margin and ventral area. In the dorsolateral area of the head capsule an anteroposterior oval depression is present, bearing two strong brown bristles, one at the anterior end of the depression and another on the back. Along the side of the capsule, a long bristle is observed, accompanied by a shorter one on the ventral side. The front clypeus and labrum form a snout, also gray; front clypeus has a lateral bristle. The antenna is a circular brown area with a whitish brown central point. In lateroventral head region there is an area not sclerotized milky-white that looks like an invagination prothorax in head capsule. Ventrally, between these two milky white areas, there is a sclerotized reddish-brown plate. The mandibles and the associated mouthparts, which are attached to this ventral plate and the dorsal snout, together form a brown complex.

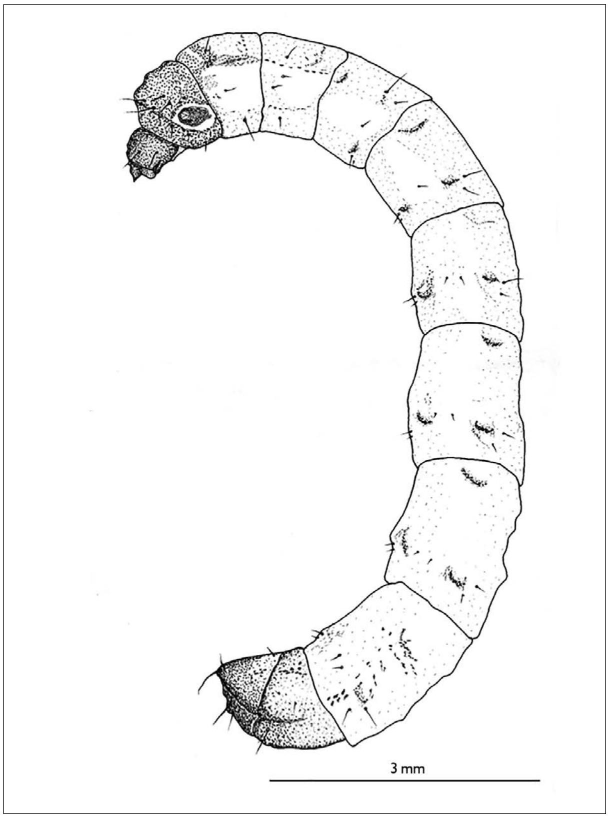

Figure 5. Second instar larva of *Pantophthalmus kerteszianus*. Illustration: I. S. Gorayeb (2014).

Thorax: In addition to the head, prothorax and the last abdominal segment are the most sclerotized. The prothorax integument is slightly sclerotized light brown, covered with coarse rugosities formed by relatively large circular wrinkles compared to other wrinkles' body. Unlike last instar, there are no rough integument dark brown sclerotized bands. Above the spiracle, dorsal area, there are two strong bristles and three other longer more queued previously. On the side there is an oval area not sclerotized, yellowish white, in the center of this area there is a smooth dark brown sclerotized spot; anterodorsal area in this spot is the spiracle round with the previous darker handle, light center two structures in the form of rods abutting the rear end, which constitute a structure in the form of 'V.' The ventral region features an unsclerotized, smooth, milky-white area lacking rugosities;

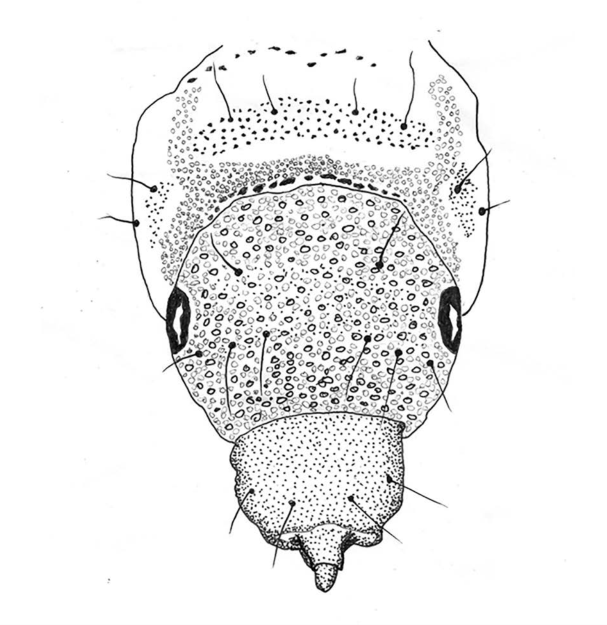

Figure 6. Head, prothorax and mesothorax of second instar larva of *Pantophthalmus kerteszianus*, dorsal view. Illustration: I. S. Gorayeb (2014).

along its upper lateral margin, a small bristle is present. Additionally, along the lateral and anterodorsal edge—where this area contacts the head capsule—there is a row of oval, dark brown sclerotized.

Mesothorax: The segment has a brown, sclerotized ring along its entire anterior margin. On each side of the body, two lateral stripes (upper and lower) are visible. In the median area between these stripes, the anterior quarter shows two areas of fine grooves and two bristles—one in the upper part and one centrally located in a dorsoventral orientation—while the remainder of the median area is milky-white. The area between the lateral stripes is slightly more swollen than other parts of milky-white segment. The upper lateral strip is sclerotized, brown, continuous, although it does not extend to the segment's edges. In contrast, the lower lateral strip is discontinuous and consists of four oval brown spots. Dorsally between the upper lateral strips on both sides of the body, mesothorax have a rugosities pattern configured by: brown areas with small circular ridges covering the leading edge and the margins of the upper strips; in the one-third previous there is an area with fine rugosities brown dark, where there are two groups of three bristles queued in the transverse direction of the segment, the central bristle in each group is greater; the posterior third there is a series of oval spots isolated, dark brown in irregular positions; later there is a finer rugosities to the posterior margin. The lower lateral area of the segment from the lower lateral strip to the venter, is milky-white and bears a bristle in the upper median area.

Metathorax: The sclerotized ring along the anterior margin is less robust than of the mesothorax. Dorsally between the lateral stripes, on each side of the body, there are no ridges, which are restricted to the anterior ring. Additionally, the upper lateral strip is weaker than the mesothorax, consisting of spots in the anterior third, while the spots on dorsoposterior are also faint. The sets of three bristles on each side of the anterodorsally area are present, the two outer bristles are closest than the mesothorax and the next middle one is smaller. In the lateral area between the upper and lower lateral strips is more whitish than the rest of the segment, in this area tegumentary weak and thin spots are present anterior and ventrally, two anteroposterior bristles are present, but the bristle is positioned more posteriorly than the lower. The lateroventral area is white-yellowish and has a brown bristle near the center anteroposterior of the lateral stripe.

First abdominal segment: It is yellowish white, with the area between the lateral stripes appearing whiter and slightly inflated. The sclerotized dark areas include: two rows of tegumentary spots stains off small, just above the upper lateral strip, a anterior, small, like a dorsoventral line formed by about ten points and another later with about eight points; tegumentary spots below the lower strip in an inflated area, where is a row of brown tegumentary points on form of inverted 'C,' structure that looks like a false leg. Bristles: Two bristles above the tegumentary line (a larger anterior and a smaller posterior) on the upper side area above the upper strip. Two bristles on the side of the strips (one upper and one lower) between spots positioned at the height of the posterior third. Two bristles in the lower lateral area below the 'false leg.'

Second to sixth abdominal segments: In these segments the color pattern, tegumentary spots and bristles are similar to those described in the 1st abdominal segment. The 5th and 6th segments have a well-defined upper lateral strip formed by 3-4 brown traces sclerotized separately in the rear area after posterior tegumentary spot; these segments there are also a number of traces sclerotized forming two lines that run along the lateral area anterior up, more evident on the 6th segment.

Seventh abdominal segment: The seventh abdominal segment appears to merge with the eighth segment, as their separation is less distinct and articulated than that observed between the other segments. Together, they form a single sclerotized structure, with a less pronounced division between them. Additionally, the upper lateral band is a fold in the integument that extends into the eighth segment, reaching its most distal point. The lower lateral stripe does not have a regular shape, it consists of 12 irregularly arranged integumentary points. The proximal third of the segment is yellowish white similar to adjacent segments, and it gradually becomes more sclerotized toward the distal margin, where the sclerotized areas are composed of circular spots. Dorsally, the distal two-thirds of the segment are sclerotized. The area between the upper and lower bands is sclerotized along its superior and posterior portions. Bristles: Pairs of are present in the three regions defined by the lateral bands—a laterodorsal pair, a median pair, and a lateroventral pair. The lateroventral area is entirely whitish except for the posterior ring.

Eighth abdominal segment: The segment is almost entirely sclerotized, except for the proximal and lateroventral areas, where sclerotization is present only as dots. The dorsum and the remaining areas are continuously sclerotized and brown, becoming darker toward the distal end. Bristles: The bristles are yellowish-brown and longer than those on the other segments: one is located dorsally, another—larger—latero-posteriorly on the lateral stripe, one is on the distal vertex, and two smaller ones are present in the middle of the lower lateral area. The anal spiracles were not described because de 'chamber' in which they are located is closed, and the larva was not dissected, since it was the only entire and preserved specimen.

REDESCRIPTION OF THE EXUVIA OF THE PUPA OF *PANTOPHTHALMUS KERTESZIANUS*

Cylindrical, body length 32 mm, wide 8 mm (Figures 7A-7C).

The anterior capsule, formed by the union of the head with the prothorax, has the anterior surface strongly chitinized and black, with a coarse rugosity. One of the dorsal wrinkles is larger and more elevated. At the anteroventral end there is a black prolongation with spines on its tip (where internally the head should be accommodated). Laterally, on the submedian and ventral region, two spines may be seen. Ventrally, on the median region there is a V-shaped structure, showing a spine on its anterior tip. The posterior area of the anterior capsule is sclerotized, smooth and shining, and brown; its margin, between the interior rugose area and this smooth area is irregular. A line separates this anterior capsule from the other segments of the thorax. In the lateroanterior region of these fused segments the thoracic spiracle is situated, being the largest one of the bodies. Dorsally and laterally, the segment exhibits several transversal grooves, oriented in a dorsoventral direction. Ventrolaterally are the wing thecae, which reach up to the anterior half of the 2nd abdominal segment. Ventrally, between the wing thecae, some tubular structures may be seen, from the anterior capsule to the 2nd abdominal segment; these structures must accommodate internally the legs and the mouthparts. The abdominal segments milky-white, and less sclerotized, showing, especially on the dorsal region, tegumentary spots. A lateral stripe is present on abdominal segments 1-7, formed by a more invaginated integument, marked by distinct upper and lower sulci. In the middle of that stripe there is a line of paler integument anteroposteriorly oriented, which is more accentuated on segments 2-5. The spiracles are clearly visible on segments 1-4, gradually decreasing in size towards the last segment. Abdominal segments 6 and 7 with rows of strong bristles on their posterior margin.

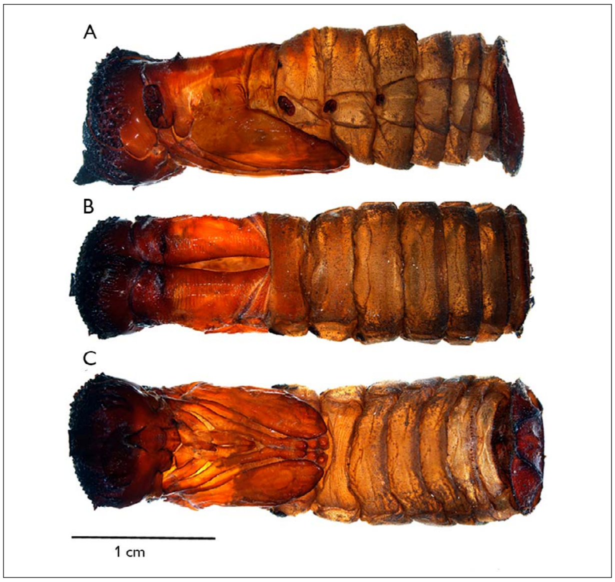

Figure 7. Pupa of *Pantophthalmus kerteszianus*: A) lateral view; B) dorsal view; C) ventral view. Images: I. S. Gorayeb (2014).

Segment 8 has a sclerotized, brown posterior plate, with two sutures dorsoventrally oriented, dividing this plate into three parts. The median part exhibits an inflated, dorsoventral region, with sclerotized rugosities. Dorsally to this structure there exists a plate shaped like a half-moon. Between this latter and the margin of the posterior plate there are rows of strong and short bristles. The anterolateral and anteroventral areas of the 8th segment display the same type of integument and color as the remaining regions, and the two abdominal spiracles are visible ventrally (Figures 7A-7C).

DIFFERENCES BETWEEN THE LARVAE OF *PANTOPHTHALMUS TABANINUS* AND *P. KERTESZIANUS*

The first instar of *P. tabaninus* is 3 mm long and 1 mm wide; the last one is 27-32 mm long and 7-10 mm wide. The last instar of *P. kerteszianus* is on average 55.33 mm long, over twice the length of that of *P. tabaninus.* The width of the larva of both species is very similar (ca. 10 mm), but the larva of *P. tabaninus* is shorter.

The morphological differences between the last instar larvae of *P. tabaninus* (Greene and Urich, 1931) and *P. kerteszianus* are shown in Table 2.

DIFFERENCES BETWEEN THE KNOWN *PANTOPHTHALMUS* PUPAE

The morphological differences between the pupae of *P. tabaninus* and *P. kerteszianus* may be seen as in Table 3.

Rapp (2007) provided a detailed description of the first instar larva of *P. planiventris*, figured in lateral and dorsal views. However, he did not specify the thoracic division nor the prothoracic spiracle. He treated the head proper together with the prothorax, that is why his drawings present, erroneously, in addition to head, 12 segments. Moreover, he mistakenly considered the prothoracic spiracles to be simple eyes. He also furnished a description of the last larval instar and the pupa, which are differentiated from those of *P. kerteszianus* by the characters presented in Tables 4 and 5 below, respectively.

Table 2. Morphological differences between the last instar larvae of *Pantophthalmus tabaninus* and *P. kerteszianus*.

Characters	*P. tabaninus*	*P. kerteszianus*
Prothorax Position of head Drawings on the spiracle	Prothorax pronouncedly curved ventrad, so that the head is downwardly directed (Plate VIII - Greene & Urich, 1931); head and prothorax with long bristles; spiracles with evident sinuous drawings	The prothorax is less curved ventrally, so that the head is directed forward. Both the head and prothorax bear shorter bristles, and the spiracles exhibit different internal pattern (Figure 1B)
Metathorax	With only one large lateroventral bristle	Meso- and metathorax with several short bristles (Figure 4)
Bristles on abdominal segments 1-7	Absent	Present and short (Figure 4)
Transversal stripes	The upper transverse stripe very evident on the mesothorax, metathorax and abdominal segments 1-7; lower transversal stripe absent	Both upper and lower transversal stripes very evident on mesothorax, metathorax and abdominal segments 1-7
Tegumentary spots	Not described, but according to the segments show darker margins and lateroventral areas	Very evident, as shown in Figure 4
Respiratory organ	Large, occupying a wide ventral area between segments 7 and 8	Small
Hair tufts at the base of the strong spines at the chitinized plate on the last segment	Not mentioned, except for the more ventral spine	Present

Table 3. Morphological differences between the pupae of *Pantophthalmus tabaninus* and *P. kerteszianus*.

Characters	*P. tabaninus*	*P. kerteszianus*
Sclerotized wrinckles of anterior capsule	Small	Larger, one of them, on the dorsal area, more projected, looking like a small horn
Spiracles	They are very visible on the thorax and abdominal segments 1-7, their internal pattern showing characteristic sinuous drawings	Visible only up to the 4th abdominal segment; different internal pattern
Fringe on segments 7 and 8	More evident, with long bristles	More discrete, with short bristles

Table 4. Morphological differences between the last instar larvae of *Pantophthalmus planiventris* and *P. kerteszianus.*

Characters	*P. planiventris*	*P. kerteszianus*
Maximum size of the larva	Length 48 mm; width 10 mm	Length 58,50 mm; width 11,0 mm
Inclination of head and prothorax in relation to the longitudinal body axis	More accentuated	Less accentuated
Shape of the body, from the mesothorax to the 7th abdominal segment	Sinuous, with a distinct waist between segments 4 and 5. From the mesothorax to the 2nd abdominal segment the segments are wider dorsocentrally. From the 3rd to the 5th the abdominal segments become gradually narrower. 6th and 7th segments respectively wider. The 5th segment is the narrowest one	Body straight, without a waist. Abdominal segments more uniform as to width
Angle formed by the sclerotized plate of the 8th abdominal segment in relation to the longitudinal axis of the body	Small	Large

Table 5. Morphological differences between the pupae of *Pantophthalmus planiventris* and *P. kerteszianus*.

Characters	*P. planiventris*	*P. kerteszianus*
Thoracic spiracle	Over twice as long as broad, not inflated. Length 2,1 mm; width 0,8 mm	Large, dark brown, noticeably inflated. Length 2,5 mm; width 1,2 mm
Abdominal spiracles	Very evident on segments 1-5	Visible only in segments 1-3. Reddish-brown. Width over twice its length
'Crown'	Proximal dorsolateral region of head with a 'crown' with a row of 11-12 sclerotized, bean-like projections, smooth and shining	'Crown' not evident. The bean-like structures reddish-brown as the integument of the thorax and not much projected
Pair of acute, black, sclerotized projections on the dorsum of the prothorax	Absent	Present
Black, sclerotized rostrum on anterolateral region	Large and protruded forwards and upwards	Smaller, less protruded
Wing thecae	Reaching the beginning of 2nd abdominal segment	Reaching the middle of 2nd abdominal segment

CONCLUSION

The second immature stages of *Pantophthalmus kerteszianus* are described for the first time, and the last larval instar and pupa are redescribed, being the only species of the family, up to now, to have the number of its larval instars determined. The descriptions are more detailed than those made for the other described immatures of the genus.

Biometric analyses revealed that *P. kerteszianus*, exhibits seven larval instars during its development, as evidenced by measurements of nine selected morphological structures. The greatest variation was observed in the body's length and width, while the smallest variation was found in the distance from the prothoracic spiracle to the lower bristle and the width of the median band of the prothorax.

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AUTHORS' CONTRIBUTION

L. E. C. Cardoso contributed to project administration, methodology, investigation, formal analysis, data curation and writing (original draft); and I. S. Gorayeb contributed to formal analysis, conceptualization, data curation, supervision, visualization and writing (review and editing).

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Reports on the ecology and behavior of Ardeidae (herons and egrets) and Threskiornithidae (Scarlet Ibis) species of a reproductive colony in a tropical estuary, São Paulo, Brazil

Relatos sobre a ecologia e o comportamento de espécies de Ardeidae (garças e socós) e Threskiornithidae (guará) em uma colônia reprodutiva em um estuário tropical no estado de São Paulo, Brasil

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Abstract: Birds from the families Ardeidae and Threskiornithidae commonly group together into intraspecific and heterospecific aggregations. In Brazil, most studies have focused on ecological and behavioral aspects related to foraging with comparatively few investigations addressing reproductive activities. This study describes a reproductive colony of these two families located in *Parque Estadual Ilha do Cardoso* on the southern coast of the State of São Paulo, Brazil. We focused on spatial use and behavioral interactions during the breeding season, and recorded six species nesting at the site. Clear differences among species were observed over three breeding seasons, including variations in reproductive period length and colony occupation patterns. Additionally, we recorded behavioral interactions such as agonistic behaviors and biparental care in the Scarlet Ibis. Notable differences in nestling and chick behavior were observed, particularly regarding nest agitation and their ability to move through vegetation. Importantly, the initial dynamic of colony occupation was marked by the arrival of a large number of Scarlet Ibis individuals. These findings underscore the importance of long-term studies to better understand the ecological relationships underlying the breeding dynamics of colonial waterbirds.

Keywords: *Ardea alba*. *Egretta thula*. *Egretta caerulea*. *Nycticorax nycticorax*. *Nyctanassa violacea*. *Eudocimus ruber*.

Resumo: Aves das famílias Ardeidae e Threskiornithidae geralmente se agrupam em agregações intraespecíficas e heteroespecíficas. No Brasil, a maioria dos estudos aborda as relações ecológicas e comportamentais apenas durante as atividades de forrageamento, e não durante as atividades reprodutivas. Diante disso, o presente estudo teve como objetivo descrever uma colônia reprodutiva dessas duas famílias (famílias Ardeidae e Threskiornithidae) localizada no Parque Estadual Ilha do Cardoso, no litoral sul do estado de São Paulo, Brasil, e descrever a organização espacial e temporal da colônia, bem como as interações comportamentais entre as espécies. Foram registradas seis espécies utilizando o local para reprodução, com variações anuais na ocupação da colônia e na duração do período reprodutivo. Também foram observadas interações comportamentais, como agonismo e o cuidado biparental entre os guarás (*Eudocimus ruber*). Entre os ninhegos, destacaram-se as diferenças comportamentais quanto à inquietação no ninho e à agilidade no deslocamento pela vegetação. Por fim, enfatizamos que a ocupação espacial e a dinâmica reprodutiva da colônia são influenciadas diretamente pela chegada dos guarás. Além disso, ressaltamos a importância de estudos de longa duração para a compreensão das relações ecológicas que fundamentam a dinâmica de reprodução destas espécies.

Palavras-chave: *Ardea alba*. *Egretta thula*. *Egretta caerulea*. *Nycticorax nycticorax*. *Nyctanassa violacea*. *Eudocimus ruber*.

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INTRODUCTION

Most species in the families Ardeidae (egrets and herons) and Threskiornithidae (ibises and spoonbills) are closely associated with aquatic environments. Many of these species use coastal ecosystem and mangroves as key areas for foraging and breeding (Custer et al., 1980; Sick, 2001). They are among the main predators of fishes and marine invertebrates in estuarine environments (Frederick, 2002; Faria et al., 2016; Dias et al., 2025).

Intraspecific and interspecific aggregations of Ardeidae and Threskionithidae are commonly observed during foraging, resting, migration, and nesting events (Kushlan & Hancock, 2005). These aggregations offer individuals protection and allow for optimal resource exploitation (Burger, 1981; Huang et al., 2021).

For most species, a key reproductive trait is their tendency to form monospecific or mixed-species colonies, with the number of species varying according to location (Kushlan & Hancock, 2005). Although the reproductive period may shift annually due to climate factors and food availability (Nachuha & Ejotre, 2014; Baker & Dieter, 2015), in Brazil these events typically begin in the austral spring (September to December) and may extend in to the austral autumn (March to June; Sick, 2001).

Colony site selection is influenced by the proximity to feeding areas, the presence of vegetation that offers nest protection, accessibility, and the characteristics of the surrounding habitat (McCrimmon Jr., 1978; van Vessem & Draulans, 1987; Frederick, 2002; Carrasco et al., 2014, 2017). Nest structures vary among species and are typically built in trees, bushes, thicket over water, and mangroves (Angehr & Kushlan, 2007; Ayala, 2022). Major limiting factors for reproductive success include nest abandonment by adults, chicks falling from nests, predation, and adverse weather condition (Frederick & Collopy, 1989; Hafner et al., 2008).

Vertical stratification of nets within colonies has been observed, generally occupying higher canopy levels and smaller species nesting in lower vegetation or understory (Burger & Gochfeld, 1990; Ayala, 2022). Furthermore, more experienced individuals tend to nest in the interior of the colony, while less experienced birds are more often found at the edges (Burger, 1981).

Although birds from the Ardeidae and Threskiornithidae families are considered relatively well-studied group, understanding their reproductive ecology remains important. Information on their breeding dynamics is essential for developing effective conservation strategies (Kushlan, 2018). Moreover, in Brazil most behavioral studies on these families have focused on foraging ecology, with relatively few addressing behavior and ecological dynamics during the reproductive period – particularly within heterospecific colonies, with emphasis on De Toledo (2000), Scherer et al. (2014), Paludo et al. (2018), Martínez et al. (2020) and Cabral et al. (2023). In this study, we describe the ecology and behavior of Ardeidae (herons and egrets) and Threskiornithidae (Scarlet Ibis) species within a reproductive colony in a tropical estuary in Brazil, monitored over three breeding seasons.

MATERIAL AND METHODS

STUDY AREA

This study was conducted in *Parque Estadual Ilha do Cardoso*, a state park that protects remnants of the Atlantic Forest biome (25° 07' 29" S and from 47° 57' 44" W; 0 m to 800 m altitudinal variation). The park is located on Cardoso Island, along the southern coast of the State of São Paulo, Brazil (Figure 1). The climate is classified as hot and humid, with average temperature ranging from 19 °C to 27 °C, and a mean annual precipitation of 2,802 mm (based on data from 2019) (CIIAGRO, 2020).

NEST CHARACTERIZATION

The bird colonies were located at the southern end of the island (25° 17' 58" S; 48° 05' 29.6" W; altitude ranging

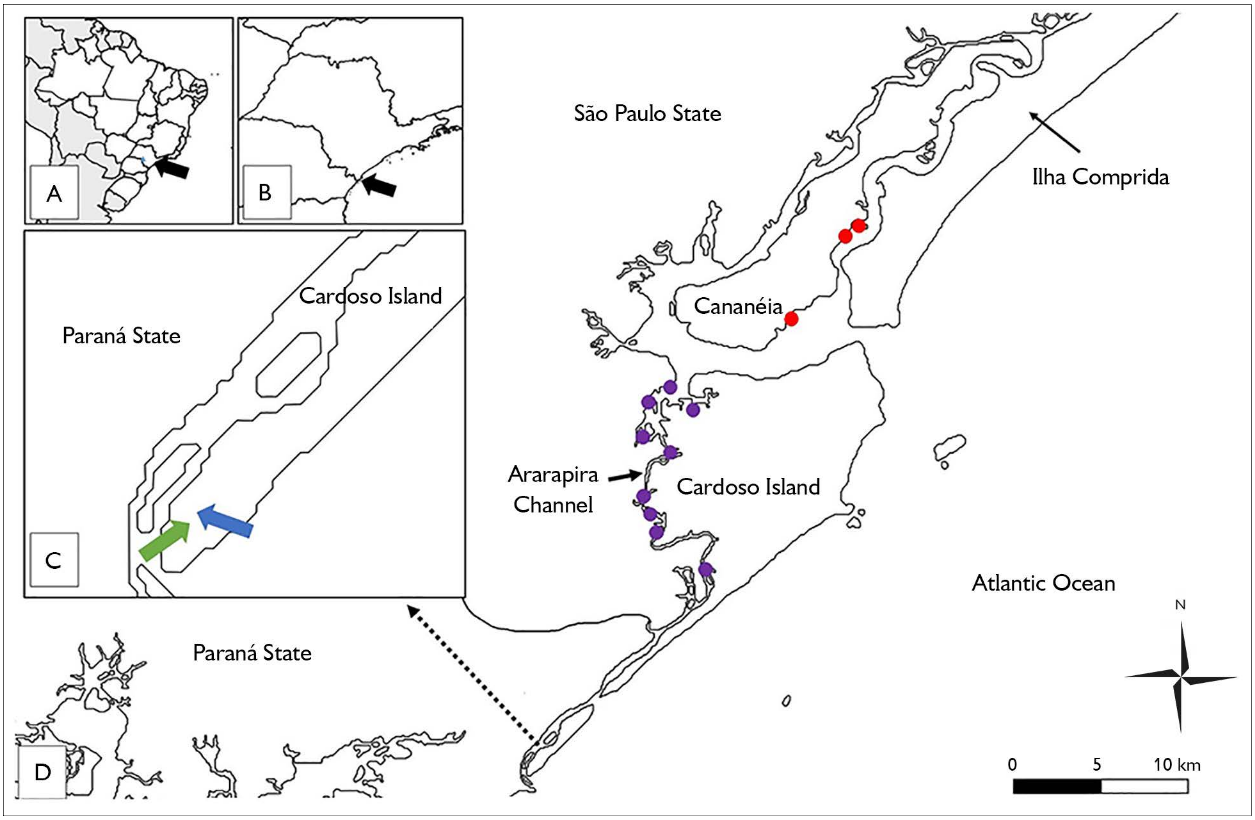

Figure 1. Geographic location of Cardoso Island on the southern coast of São Paulo State (A, B), Brazil. (C) Green arrow indicates the nesting colony area; blue arrow marks the Pontal do Leste Community. Red points represent foraging areas in Cananéia, while purple points indicate foraging areas along the Ararapira Channel (D). Map: Henrique Chupil (2025).

from 0 m to 5 m), near the Pontal do Leste Community. The site is bordered to the west by the Ararapira Channel and to the east by the Atlantic Ocean (Figure 1). The area encompasses approximately 20,000 m^2. Water volume changes according to the tides (Figure 2), with tidal inflow entering the lagoon through a stream connected to the Ararapira Channel.

The lagoon is surrounded by three mangrove species: *Avicenia schaueriana* Stapf & Leechm. ex Moldenke, *Rizophora mangle* L., and *Laguncularia racemosa* (L.) C.F. Gaertn. (Figure 2B). A sand strip separates the lagoon from the Atlantic Ocean, which is vegetated by *Hibiscus pernambucensis* Arruda, *Psidium cattleyanum* Afzel. ex Sabine, *Conocarpus erectus* L., and other shrub species, along with marsh areas dominated by *Typha latifolia* L. (Cunha-Lignon et al., 2011). The distance between the beach and the lagoon varies from 50 to 70 meters.

The identification of the reproductive species present in the colonies was conducted during the first field expedition.

DATA SAMPLING

Fieldwork was conducted by one to four researches who traveled by the boat from the city of Cananéia between September and May during the 2015/2016, 2016/2017 and 2017/2018 breeding seasons, with monthly expeditions (averaging one day per month). Behavioral observations were carried out from a sand strip located to the south of the lagoon, which surrounds the nesting area. We employed naturalistic observation using scan sampling (Altmann, 1974) to minimize disturbance to bird behavior, aided by binoculars (Bushnell 10x42). To study individual behavior and social interactions, both focal animal sampling and sequential sampling methods were used, allowing for the recording of one or more behaviors from multiple individuals. Photograph records were obtained with a Nikon camera D3300 (lens 70-300 mm) and Canon 5D Mark II (lenses 100x500 mm and 100x400 mm).

Visual assessments of bird abundance were conducted by surveying the outer perimeter of the nesting area. Egg counts were performed by approximating nest contents in accessible areas, limited to nests located up to two meters high.

To map the spatial distribution of individuals and nests within the lagoon, we conducted three short Unmanned Aerial Vehicle (UAV – DJI/Mavic) flights, each lasting 10–12 minutes, all on the same day. In addition, we periodically used a kayak to navigate within the lagoon, providing alternate visual access to the nesting area. Both UAV flights and kayak incursions maintained a minimum distance from the birds to avoid behavioral disturbance.

The identification of foraging areas beyond the Ararapira Chanel was carried out during the monthly boat

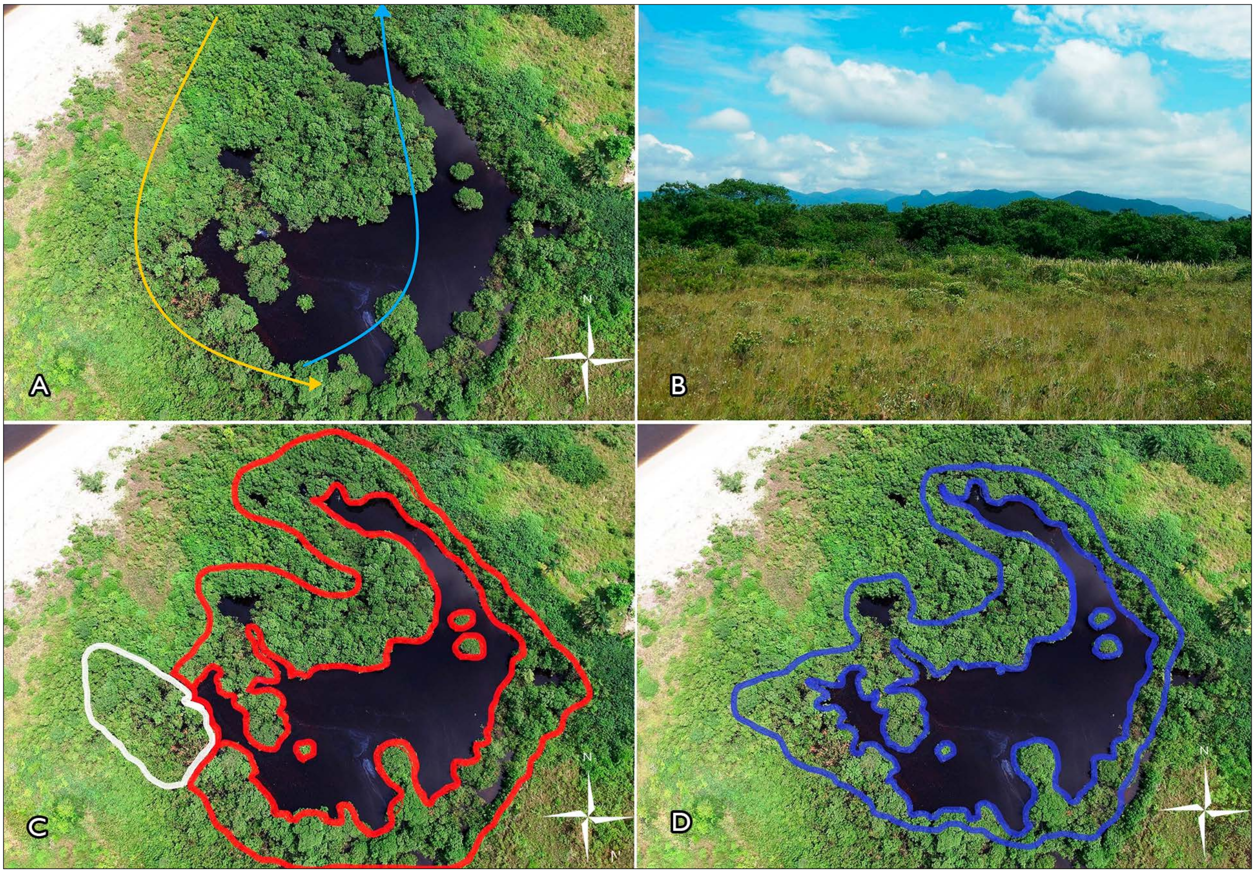

Figure 2. Nesting colony area located in the southern part of Ilha do Cardoso: A) aerial image of the lagoon: the yellow arrow indicates the flight path of birds arriving at the nesting colony, while the blue arrow indicates the path of birds departing the area; B) frontal view of the nesting colony, with shrubby vegetation in the foreground and typical mangrove species in the background surrounding the lagoon; C) distribution of the reproductive area (red polygon) and the resting/dormitory area (white polygon) during the breeding seasons from September 2015 to May 2016 and September 2017 to April 2018; D) distribution of the reproductive, dormitory, and resting areas (blue polygon) during the breeding season from September 2016 to February 2017. Images: Authors (2016).

expeditions to the reproductive colony. In Cananéia, the foraging areas were located based on monthly incursions along the center island coast. Although the birds were not banded, we inferred their use of these areas based on consistent flight, assuming that individual breeding in the colony regularly moved to these sites.

In the case of the Scarlet Ibis, it was possible to distinguish between males and females, as males typically have bills that are, on average, 22% longer than those of females and exhibit darker coloration (Hancock et al., 1992; Sick, 2001).

RESULTS

BIRD SPECIES RECORDED AND SPATIAL OCCUPATION OF NESTING AREA

We recorded six bird species breeding within the study area – five belonging to the family Ardeidae: Black-crowned Night-Heron, Yellow-crowned Night-Heron, Snowy Egret, Little Blue Heron, and Great Egret; and one species from the family Threskiornithidae: Scarlet Ibis (some examples are shown in Figure 4). Only two Great Egret nests were recorded, both observed in September 2016; located at the top of a tree on a small, isolated island within the lagoon.

The first species to arrive at the colony at the beginning of the breeding season were the Black-crowned Night-Heron and Yellow-crowned Night-Heron, followed by the Snowy Egret, Little Blue Heron, and Scarlet Ibis. Across all three reproductive seasons, Scarlet Ibis was the most abundant species in the colony (Table 1). Black-crowned Night-Heron, Yellow-crowned Night-Heron, Snowy Egret, and Great Egret were more abundant during the 2016/2017 and 2017/2018 seasons, whereas Little Blue Heron was most abundant in 2015/2016 (Table 1).

During two of the reproductive seasons, Scarlet Ibises used separate areas within the site as resting (for both breeding and non-breeding individuals) and dormitory zones (non-breeding birds only). During the incubation period, adults typically rested near their nests. Nest distribution covered a broad area of the site, while resting/dormitory areas were more spatially defined in the 2015/2016 and 2017/2018 seasons (Figure 2C). In contrast, during the 2016/2017 breeding season, nests were more concentrated in the inner portion of the lagoon, overlapping with the area previously used for resting/dormitory activities (Figure 2D). A distinct resting/dormitory area was not identified during that season. For the other species, no specific resting or dormitory zones were recorded.

Throughout the three breeding seasons, nests of Snowy Egret and Little Blue Heron were commonly located along the edge vegetation of the lagoon, especially in 2016/2017. In contrast, Black-crowned Night-Heron and Yellow-crowned Night-Heron consistently nested in elevated locations within dense vegetation farther from the lagoon, showing a stable nest placement pattern across seasons.

Table 1. Estimated average number of birds (September–May) per breeding season.

Species	Breeding Seasons		
	2015/2016	2016/2017	2017/2018
Black-crowned Night-Heron	18	70	50
Yellow-crowned Night-Heron	12	30	25
Snowy Egret	19	23	20
Little Blue Heron	30	16	15
Great Egret	1	4	2
Scarlet Ibis	1,000	400	900

Although nest construction followed a general pattern—interwoven bundles of branches—Scarlet Ibis, Black-crowned Night-Heron, and Yellow-crowned Night-Heron nests were composed of a greater number of branches. These structures appeared to be more resistant to weather, as evidenced by the low number of damaged nests observed in the subsequent breeding season.

FORAGING BEHAVIOR AND FLIGHT PATTERNS

Across all three seasons, all recorded species foraged predominantly along the Ararapira Channel, at distances ranging from 1 km (mangrove zones) to 26 km (mudflats) from the colony (Figure 1). In other seasons, birds also foraged on mudflats located on the southeastern and eastern parts of Cananéia Island, up to 40 km from the nesting site (Figure 1). Within the lagoon, foraging was recorded during low and ebb tides, when the muddy edges became exposed. These conditions allowed foraging by Little Blue Heron, juvenile Scarlet Ibises, and both species of night-herons. Other species continued to forage in shallow waters within the lagoon.

At sunrise, most adult Scarlet Ibises dispersed individually or in small groups to foraging areas to the north. At these sites, group sizes could reach up to 300 individuals (based on counts conducted in December 2018). Throughout the day, small groups (2 to 5 individuals) regularly returned to the colony to feed chicks, while others simultaneously departed for feeding grounds. By sunset, large flocks—sometimes up to 30 birds—returned to the colony to roost. During the 2015/2016 breeding season, the high density of Scarlet Ibises resulted in a shortage of suitable perching sites, leading some individuals to abandon the colony and fly westward toward the Paraná State border.

Scarlet Ibis flocks typically flew in a V-formation, a pattern commonly observed in aquatic bird species (Hummel, 1983). Most flocks approached the colony from the north via the west side, performing counterclockwise circular descents above the colony to reduce wind resistance from prevailing eastern and southern sea winds. At lower altitudes and reduced speed, individuals could safely perch on vegetation. When departing the colony, most birds first flew over the lagoon, then ascended and continued northward. This allowed us to identify distinct arrival and departure routes for the nesting area (Figure 2A).

REPRODUCTION

The longest reproductive season was recorded in 2015/2016, spanning nine months from August to May. In contrast, the 2016/2017 season lasted five months (September to February), and the 2017/2018 season extended for seven months (September to April) (Figure 3).

During the first monitored breeding season (2015/2016), nest-building began in August, and nests containing eggs were already present during the first field expedition in September. In the subsequent seasons, the earliest nest-building activity was observed in September and was initiated by the Yellow-crowned Night-Heron and Black-crowned Night-Heron. Other species began nest construction only from November onward (Figure 3). In all three seasons, egg-laying commenced immediately following nest construction.

Nestlings were observed from September to April during the 2015/2016 season, from October to February in 2016/2017, and from October to March in 2017/2018 (Figure 3). The presence of chicks was recorded from September to March in 2015/2016, and from October to February in both 2016/2017 and 2017/2018 (Figure 3). A reproductive season was considered concluded when no eggs, nestlings, or chicks were observed in the colony. These endpoints occurred in May 2016, February 2017, and April 2018, respectively.

Across all species, the number of eggs per nest ranged from one to four. The average number of nestlings per nest was two for Scarlet Ibis and three for Yellow-crowned Night-Heron, Black-crowned Night-Heron, Snowy Egret, and Little Blue Heron. Due to the dense vegetation and the limited frequency of incursions into the nesting area, it was not possible to estimate the total number of nests per species in the colony.

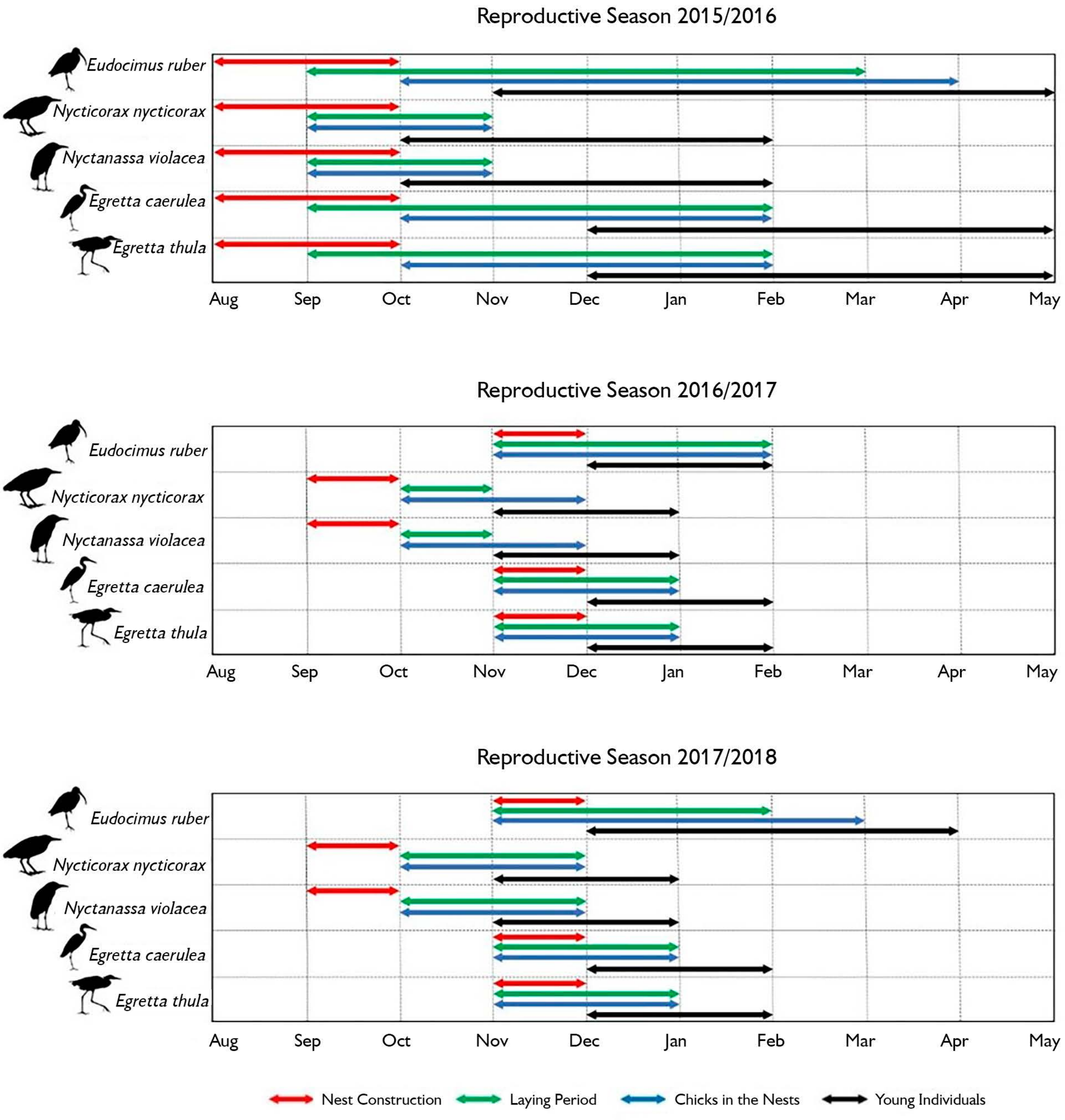

Figure 3. Periods of nest construction (red arrows), egg-laying and incubation (green arrows), presence of chicks in nests (blue arrows), and presence of young outside the nests (black arrows) during the 2015/2016, 2016/2017, and 2017/2018 breeding seasons. Image: Henrique Chupil (2025).

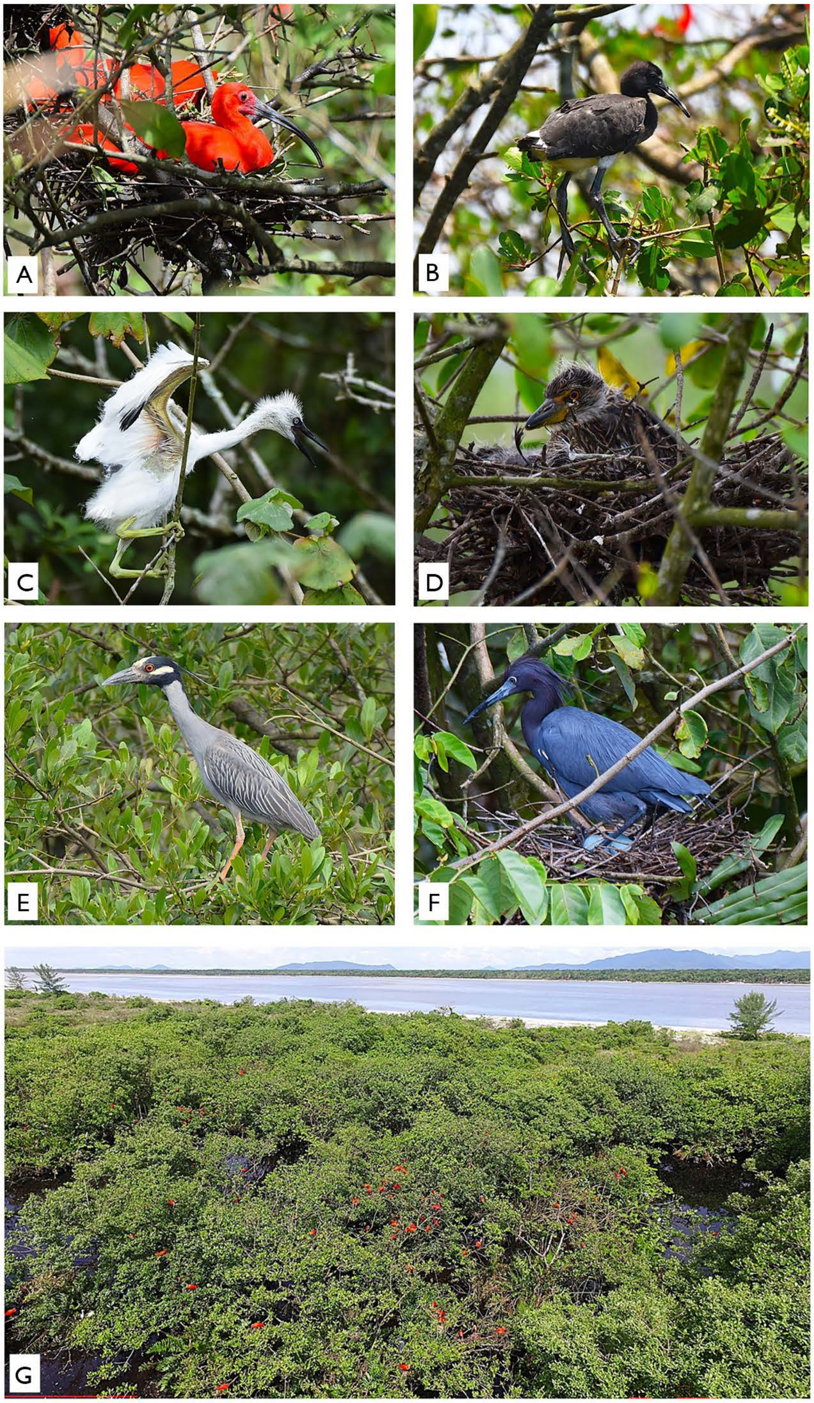

Figure 4. A) Scarlet Ibis at the nest; B) Scarlet Ibis chick; C) Little Blue Heron chick; D) Blackcrowned Night-Heron chick; E) Yellow-crowned Night-Heron; F) little Blue Heron at the nest; G) aerial image of Scarlet Ibises within the colony. Images: Authors (2016).

BEHAVIORAL INTERACTIONS

Few behavioral interactions were observed among the species and among the specimens, and these occurred only occasionally, typically in relation to competition for landing sites. Snowy Egret exhibited agonistic behavior primarily in response to direct pressure. Little Blue Heron were the most behaviorally active. Scarlet Ibis interactions were relatively rare and more discreet compared to other species. Most disputes were intraspecific, generally involving competition for nesting sites within the colony or for materials used in nest construction.

Two noteworthy reproductive behaviors were documented for Scarlet Ibis: (1) two different females feeding chicks in the same nest, and (2) biparental care. During the incubation period, males typically assumed responsibility for nest duties during the morning hours, while females remained on the nest during the afternoon. As nestlings approached the fledging stage, both males and females would leave the nest to forage.

Scarlet Ibis, Black-crowned Night-Heron, and Yellow-crowned Night-Heron showed no observable reaction to potential predators such as the Southern Crested Caracara (*Caracara plancus* (Miller, 1777)) and the Great Black Hawk (*Urubitinga urubitinga* (Gmelin, 1788)). Furthermore, no significant behavioral responses were recorded in reaction to UAV flights over the nesting areas. Among all species, only Scarlet Ibis demonstrated greater tolerance to human presence during egg incubation. In contrast, egrets were more sensitive to disturbance, leaving their nests when approached by humans or during UAV operations.

BEHAVIOR OF NESTLINGS AND CHICKS

Nestlings of all species exhibited similar behaviors, remaining mostly inactive and becoming active when adults returned to feed them. Little Blue Heron and Snowy Egret nestlings had difficulty moving through the vegetation and generally stayed close to the nest. Scarlet Ibis nestlings, in contrast, were more developed and moved through and above the vegetation with greater agility. Yellow-crowned Night-Heron and Black-crowned Night-Heron nestlings remained mostly within their nests, which were more robust than those of the other species.

In the presence of potential predators, all nestlings displayed defensive behavior by standing motionless in an upright position.

Chicks exhibited species-specific behavioral differences. Black-crowned Night-Heron and Yellow-crowned Night-Heron chicks were predominantly slow-moving or motionless, mirroring the behavior of adults. Snowy Egret and Little Blue Heron chicks were more active and engaged in more exposed behaviors. Scarlet Ibis chicks displayed similar levels of activity but with more agile movements, often using their wings to assist in leaping between branches. This behavior intensified when adults approached with food, occasionally resulting in short chases through the vegetation and brief flights.

Chicks were also observed attempting to feed from non-parental adults, though these attempts were unsuccessful. Toward the end of chick development, some individuals—after being fed—continued to chase their parents for additional food. In response, adults would leave the nest, fly up to 200 meters away, and return, still pursued by their chicks. During early flight attempts, the juveniles showed irregular and unstable flight, which gradually improved over time.

Across all species, chicks typically remained still or made only minor movements, with significant activity occurring primarily during adult feeding visits.

Before dispersing from the nesting colony, Scarlet Ibis chicks formed small groups and made short flights around the area. After leaving the colony, they remained grouped, primarily on nearby mudflats off Cananéia Island. For the other species, departure from the colony was gradual and occurred as the chicks became capable of sustained flight.

DISCUSSION

The breeding species recorded in the colony are typical of estuarine environments in the Parque Estadual Ilha do Cardoso (Chupil & Monteiro-Filho, 2022) and the municipality of Cananéia (Chupil, 2019). However, breeding activities in the region are noorly documented, with notable contributions by Noguchi (2015) and Paludo et al. (2018). Residents of a local village (Pontal do Leste) near the colony report that the nesting area was often used by a large number of egrets and herons as nesting and dormitory sites before 2014. Only in 2014 Scarlet Ibis started to visit and used the site for resting and breeding. Our records of six actively breeding bird species in the same nesting area reflect the typical colonial behavior described for most species in the Ardeidae and Threskiornithidae families (Kushlan & Hancock, 2005). During the 1900s, there was a significant decline in records of the Scarlet Ibis along the southern and southeastern coasts of Brazil. It was only in the early 1980s that Scarlet Ibis numbers gradually began to increase (Chupil & Monteiro-Filho, 2018). Reports of breeding events came eight years after the Scarlet Ibis was first sighted in the municipality of Cananéia (Chupil & Monteiro-Filho, 2018). The presence of Scarlet Ibises likely induced changes in the previously dynamic spatial use by other species, as residents near the nesting area reported that egrets and herons were predominant nesters before 2014 (Wellington das Neves, personal communication, 2015). With the arrival of the Scarlet Ibis, the number of egrets nesting within the core area of the colony declined. Instead, egrets began nesting in the peripheral areas of the lagoon, a pattern we observed consistently across all three reproductive seasons. Similarly, Hass et al. (1999) at Ilha do Cajual (State of Maranhão, northeastern Brazil) and Olmos and Silva-Silva (2001) at Cubatão (State of São Paulo) reported a predominance of Scarlet Ibis nesting over other species in mixed colonies.

The length of the reproductive period varied across the monitored seasons. The 2015/2016 season was the longest, beginning as early as austral winter. In other seasons, herons started breeding in September, while other species began in November. The reproductive timing observed in the last two seasons seems to reflect a common pattern among most colonial bird species nesting in southern and southeastern Brazil. Similar reproductive period lengths have been documented by other researchers for Scarlet Ibis (Olmos & Silva-Silva, 2001, 2003; Grose, 2016), egrets (Olmos & Silva-Silva, 2002; Noguchi, 2015), and herons (Branco & Fracasso, 2005; Grose et al., 2014). The extended length of the 2015/2016 season may be associated with the recent arrival of the Scarlet Ibis to the colony. Most species were likely still adapting to new conditions imposed by the high density of Scarlet Ibis and possible environmental variations, both of which could influence bird physiology.

The dominance of Scarlet Ibis nests and their occupation of a wider area has previously been described by Olmos and Silva-Silva (2003) in the mangroves of Santos and Cubatão (southeastern Brazil), where high nesting density directly contributed to reproductive success. This behavior is primarily driven by the reduction in predation risk (Hamilton, 1971; Moraes & Krul, 1995), a strategy also effective for other colonial nesting species (Kushlan & Hancock, 2005). The earlier arrival of herons to the colony may reflect their preference for more sheltered nesting sites, as early-arriving species often have priority in site selection (Burger & Gochfeld, 1985; Kim & Koo, 2009).

Proximity to feeding areas is often considered a key factor in the selection of nesting colony sites (McCrimmon Jr., 1978; Frederick, 2002). In this study, during the reproductive period, most birds were observed foraging primarily in the Ararapira Channel, located 1-26 km from the colony. In contrast, after the reproductive period, a greater number of birds were observed foraging on mudbanks to the southeast and east of Cananéia Island, at distances ranging from 40 km from the colony

The alternating parental care observed in Scarlet Ibis during nest building, egg incubation, and chick rearing aligns with the general behavioral patterns described for the Ardeidae and Threskiornithidae families (Frederick, 2002). However, in this study, we observed greater parental segregation during incubation, with males tending to incubate more frequently in the morning and females more in the afternoon. We also recorded alloparental care in chick feeding, likely performed by individuals that either did not reproduce that year or lost their offspring, as previously documented for the American White Ibis (*Eudocimus albus* Linnaeus, 1758) by Herring and Gawlik (2007).

We observed that adult Scarlet Ibises, when landing to feed their young, often did so at a distance, prompting the chicks to pursue them for food. Recently fledged chicks would chase through the branches and occasionally rely on their wings for support. This behavior is similar to Hoatzin (*Opisthocomus hoazin* Statius Muller, 1776) chicks, specie with claws on the tips of its wings (Sick, 2001). Over time, they began making short flights until they were capable of fully leaving the nest. We believe this behavior was selected to stimulate flight development in the young, gradually improving their motor skills and flight capabilities. The flight's incentive for rapid emancipation to youngs was observed in White-Ibis by Petit and Bildstein (1986), being related with increase survival chances outside the colony.

Behavioral interactions within the colony were generally discreet for most species, except for egrets. Agonistic behaviors are commonly observed among egrets during foraging, both intra- and interspecifically (Coelho, 2009), often linked to the defense of feeding sites (Moralez-Silva et al., 2010). In this study, we also recorded such interactions in a reproductive context, which may help explain the spatial segregation of egret nests. Scarlet Ibises exhibited only sparse agonistic interactions, primarily intraspecific, mostly involving competition for landing spots, as also reported by Vigário (2014) in the state of Paraná. The low incidence of conflict among individuals may be attributed to the dense use of vegetation and the close proximity of nests, both of which contribute to the reproductive success of the species.

In summary, the initial occupation of the colony by a large number of Scarlet Ibis individuals led to changes in colony dynamics and directly affected the abundance and spatial distribution of other species. This highlights the importance of long-term studies to better understand the ecological relationships underpinning the reproductive dynamics of colonial birds and to inform more effective management actions. Conservation measures for the studied colony should include the protection of mudbanks, which serve as essential foraging areas, and the regulation of tourism to minimize disturbances to bird activity both at the colony and foraging sites.

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AUTHORS' CONTRIBUTION

H. Chupil contributed to project administration, formal analysis, funding acquisition, conceptualization, data curation, investigation, methodology, resources, software, supervision, validation, visualization, and writing (original draft, review, and editing); C. N. Louzada contributed to investigation, resources, validation, visualization, and writing (review and editing); and E. L. A. Monteiro-Filho contributed to formal analysis, conceptualization, data curation, investigation, methodology, supervision, validation, visualization, and writing (review and editing).

Notas de pesquisa

Area of occurrence extension of *Astyanax courensis* Bertaco, Carvalho & Jerep, 2010, and *Psalidodon goyanensis* (Miranda-Ribeiro, 1944) (Characiformes: Acestrorhamphidae) with new records from tributaries in the States of Goiás and the Federal District, Brazil

Ampliação da área de ocorrência de *Astyanax courensis* Bertaco, Carvalho & Jerep, 2010, e *Psalidodon goyanensis* (Miranda-Ribeiro, 1944) (Characiformes: Acestrorhamphidae), com novos registros provenientes de tributários nos estados de Goiás e do Distrito Federal, Brasil

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Abstract: In this study, we report new records of two freshwater fish species: *Astyanax courensis* and *Psalidodon goyanensis*. Both species are considered endemic to the Upper Tocantins river basin, with their known distribution originally restricted to the Chapada dos Veadeiros region in the State of Goiás, Brazil. The results of this study expand the known range of these species by documenting their presence in sub-basins located in both the Federal District and the State of Goiás, within the limits of the Upper Tocantins basin.

Keywords: *Cerrado*. Chapada dos Veadeiros. Endemism. Freshwater fishes. Upper Tocantins River.

Resumo: Neste estudo, relatamos novos registros de duas espécies de peixes de água doce: *Astyanax courensis* e *Psalidodon goyanensis*. Ambas são consideradas endêmicas da bacia do alto rio Tocantins, com ocorrência originalmente restrita à região da Chapada dos Veadeiros, no estado de Goiás, Brasil. Os resultados obtidos neste trabalho ampliam o conhecimento sobre a área de ocorrência dessas espécies, revelando sua presença em sub-bacias localizadas no Distrito Federal e em Goiás, ainda dentro dos limites da bacia do alto Tocantins.

Palavras-chave: Cerrado. Chapada dos Veadeiros. Endemismo. Peixes de água doce. Alto rio Tocantins.

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INTRODUCTION

The *Cerrado* is a Brazilian biome that holds significant importance as a water resource, often referred to as the "cradle of waters" (Oliveira et al., 2020). The elevated regions of this biome play a crucial role in bearing the headwaters of several river systems (Codeplan, 2020). Within the Federal District, the headwaters of three major South American basins originate: Tocantins (of the Tocantins-Araguaia rivers basin, part of the Amazon drainage), Upper Paraná, and São Francisco rivers. From those, 13% of the Federal District's territory is permeated by the Tocantins basin (Sawyer et al., 2017; Codeplan, 2020). Recent studies highlight the remarkable fish diversity in the Tocantins-Araguaia basin, which harbors around 750 valid fish species (Chamon et al., 2022; Araújo et al., 2025). However, along stretches of the Upper Tocantins basin, there is a noticeable expansion of land use, including agricultural activities and human occupation, resulting in significant loss of *Cerrado* landscapes (Araújo et al., 2025). These environmental changes negatively affect water bodies and detrimentally impact fish populations (McFadden et al., 2022).

Among the Tocantins-Araguaia basin, Characiformes represent the most diverse fish order, comprising 40.3% of the known fish diversity (Chamon et al., 2022). Recently, the formerly recognized Characidae (previously the most diverse characiform family) was divided into four families: Spintherobolidae, Stevardiidae, Characidae (redefined), and Acestrorhamphidae (Melo et al., 2024). Among those, Acestrorhamphidae, with 15 subfamilies, is now the most diverse family, including part of what is currently recognized as *Astyanax* (Baird et al., 1854; Melo et al., 2024).

The genus *Astyanax* was originally described to include small freshwater characids from Central and South America and eventually became an extremely broad genus (Baird et al., 1854; Ornelas-García et al., 2008), reaching a total of 272 species, with currently 125 valid species being recognized (Fricke et al., 2025). Through time, the absence of exclusive characters to delimit *Astyanax* led to the formation of artificial groupings within the genus, thereby bringing together unrelated lineages that shared some external resemblance (e.g., Lima et al., 2003; Mirande, 2010; Terán et al., 2020). In this context, some genera were synonymized under *Astyanax*, such as *Psalidodon* Eigenmann (1907), since the characters originally used to distinguish *Psalidodon* were later interpreted as variations of *Astyanax*, insufficient to delimit clades (Lima et al., 2003). Consequently, *Psalidodon* species came to be included within *Astyanax* (Lima et al., 2003; Mirande, 2010). However, more recently, based on morphological and molecular evidence, Terán et al. (2020) resurrected the genus *Psalidodon*, reallocating to it several species previously assigned to *Astyanax*. This taxonomic revision was later corroborated by the phylogenomic analyses conducted by Melo et al. (2024).

The delimitation proposed by Eigenmann (1917, 1921) for *Astyanax* was, for a long time, widely accepted, and used to assign species to this genus. This definition was based on the presence of two series of teeth on the premaxilla, with the second series generally composed of five teeth; denticulated crowns on the premaxillary and dentary teeth; maxilla bearing 0 to 9 teeth; a complete lateral line; the absence of a predorsal spine; and the absence of scales on the caudal fin (Eigenmann, 1921, 1971). However, most of these characters are broadly distributed among various tetra fish and piaba, and are not exclusive to *Astyanax*, highlighting the need for a redefinition of the genus through phylogenetic studies (Mirande, 2010; Terán et al., 2020).

The redefinition of the genus *Psalidodon* proposed by Terán et al. (2020) was based on an exclusive combination of derived morphological characters that distinguish it from other genera. The genus is diagnosed by the lateroventral orientation of the anterior laterosensory pore to the dilatator fossa; absence of a dorsal projection of the rhinosphenoid between the olfactory nerves; absence of circuli on the posterior portion of the scales; presence of an anterior branch in the maxillary tubules; presence of 0–3 maxillary teeth (generally one); origin of the anal fin located posterior to the vertical line passing through the last dorsal-fin ray; and absence of scales on the caudal fin (Terán et al., 2020). This exclusive combination of characters, altogether with molecular evidence, support *Psalidodon* and confirm its distinction from the genus *Astyanax*, which was later corroborated by phylogenomic analyses (Melo et al., 2024).

In this study, we address the extension of occurrence of two species of Acestrorhamphidae currently assigned to the genera *Astyanax* and *Psalidodon*. *Astyanax courensis* (Bertaco et al., 2010) is currently considered endemic to the Upper Tocantins river basin. Its occurrence was originally recorded in tributaries of the Rio dos Couros, particularly in the Chapada dos Veadeiros region, Goiás State. The type-locality designated for *Astyanax courensis* corresponds to the Rio dos Couros, located in the Parque Portal da Chapada, a tributary of the Tocantinzinho river, which belongs to the Tocantins river basin (Bertaco et al., 2010). This species is distinguished from other congeners by presenting an exclusive combination of characters, being two humeral spots, a caudal peduncle spot; 35–36 lateral-line scales; three tricuspid to pentacuspid teeth in the outer premaxillary series and five pentacuspid teeth in the inner series; eight branched dorsal-fin rays; 11–13 branched anal-fin rays; and seven pelvic-fin rays (Bertaco et al., 2010).

Psalidodon goyanensis (Miranda-Ribeiro, 1944), originally described as *Astyanax goyanensis*, was transferred to *Psalidodon* by Terán et al. (2020) based on morphological and molecular data. Its occurrence was originally recorded in the Rio dos Couros, in the Chapada dos Veadeiros region, as well as in the Tocantinzinho and Paranã rivers (Bertaco et al., 2010). *Psalidodon goyanensis* is distinguished from other congeners by the exclusive combination of characters: presence of a single rounded humeral spot located just posterior to the opercle; horizontally elongated caudal peduncle spot, reaching the tip of the middle caudal-fin rays; complete lateral line, with 35–37 perforated scales; dorsal fin with two unbranched and eight branched rays; anal fin with three unbranched and 13–15 branched rays; and pelvic fins with one unbranched and 7 branched rays (Bertaco et al., 2010). To differentiate *P. goyanensis* from *A. courensis*, two similar species occurring in the same streams, the most notable characters are the number of pelvic-fin rays (seven in *P. goyanensis* and eight in *A. courensis*), as well as differences in the number of lateral-line scales and the pattern of humeral spots (two humeral spots in *A. courensis*, and one in *P. goyanensis*) (Bertaco et al., 2010).

Understanding the geographic distribution of these species is essential for elucidating their patterns of diversity (Albert & Reis, 2011). Although studies have been conducted on tributaries of the Paraná basin in the Federal District (e.g., Aquino et al., 2009), research on the ichthyofauna of tributaries of the Tocantins river basin remains scarce, possibly because this basin occupies a smaller portion of the region. However, recent collections, carried out mainly between 2021 and 2023, captured specimens of *A. courensis* and *P. goyanensis* outside their previously known distribution ranges. In this context, the present study expands the area of occurrence of *A. courensis* and *P. goyanensis* in the Upper Tocantins river basin.

MATERIAL AND METHODS

The data analyzed were obtained from specimens harbored in the Ichthyological Collection of the University of Brasília (CIUnB), collected between 2006 and 2023. At CIUnB, 12 lots comprising 269 specimens of *A. courensis* and 8 lots with 148 specimens of *P. goyanensis* were analyzed. Specimens of *A. courensis* and *P. goyanensis* were recorded in several localities of the Upper Tocantins River basin (Table 1). Both species were identified according to their original descriptions (Bertaco et al., 2010; Miranda-Ribeiro, 1944) and subsequent updates (Terán et al., 2020; Melo et al., 2024). The morphometric data used for the proper delimitation of the species were organized and are presented in the Table 2, following the procedures proposed by Bertaco et al. (2010).

Table 1. Localities of lots of *A. courensis* and *P. goyanensis* deposited at CIUnB.

Region	Catalog number	Watercourse	Geographic coordinate
Astyanax courensis			
Distrito Federal, Brasília	CIUnB 533	Dois Irmãos Stream	15.55050° S, 48.10580° W
Distrito Federal, Brasília	CIUnB 513	Palma River	15.51487° S, 48.06089° W
Distrito Federal, Fercal	CIUnB 541	Buraco Stream	15.59683° S, 47.90883° W
Distrito Federal, Fercal	CIUnB 139	Maranhão River	15.59430° S, 47.91675° W
Distrito Federal, Fercal	CIUnB 868	Taquari Stream	15.57313° S, 47.93952° W
Distrito Federal, Fercal	CIUnB 555	Cafuringa Stream	15.54164° S, 47.96622° W
Distrito Federal, Planaltina	CIUnB 837	Vereda Grande Stream	15.54216° S, 47.57801° W
Distrito Federal, Planaltina	CIUnB 2267	Brejo Stream	15.54018° S, 47.68743° W
Distrito Federal, Planaltina	CIUnB 2312	Maranhão River	15.51613° S, 47.69635° W
Distrito Federal, Brazlândia	CIUnB 1941	Poço Azul	15.55800° S, 48.04400° W
Distrito Federal, Sobradinho	CIUnB 1450	Salinas Morro da Pedreira Stream	15.51441° S, 47.95142° W
Goiás, Planaltina	CIUnB 1051	Contendas Stream	15.46805° S, 47.64161° W
Psalidodon goyanensis			
Distrito Federal, Brasília	CIUnB 128	Buraco Stream	15.59885° S, 47.89685° W
Distrito Federal, Planaltina	CIUnB 840	Vereda Grande Stream	15.52037° S, 47.56781° W
Distrito Federal, Sobradinho	CIUnB 1768	Ouro Stream	15.51200° S, 47.93000° W
Goiás, Cavalcante	CIUnB 1375	Conceição River	13.63500° S, 47.83826° W
Goiás, Cavalcante	CIUnB 1355	Mirante Stream	13.63241° S, 47.81905° W
Goiás, Cavalcante	CIUnB 1362	Santa Rita Stream	13.61496° S, 47.84793° W
Goiás, Cavalcante	CIUnB 1358	Santa Rita River	13.60140° S, 47.84300° W
Goiás, Cavalcante	CIUnB 1503	São Félix River	13.56268° S, 47.71522° W

Table 2. Morphometric data of analyzed specimens of *Astyanax courensis* and *Psalidodon goyanensis*. Legends: Min = minimum; Max = maximum; SD = standard deviation; *n* = number of analyzed specimens; SL = standard length.

	Astyanax courensis				*Psalidodon goyanensis*			
	Min	Max	SD	*n*	Min	Max	SD	n
Standard length (mm)	12.22	79.96	0	83	19.4	60.93		51
	As percentage of SL							
Predorsal distance	49.2	59.7	2.1	81	45.5	59.5	2.7	51
Prepelvic distance	32.1	57.6	3.4	81	42.4	55.6	2.4	51
Prepectoral distance	17.9	34.1	2.4	81	24.3	33.4	2.0	51
Preanal distance	60.5	72.0	2.1	81	61.7	77.5	2.9	51
Depth at dorsal-fin origin	22.5	43.6	4.0	81	26.6	36.6	2.3	51
Caudal-peduncle depth	7.7	25.3	1.9	81	9.5	20.9	2.6	51
Caudal-peduncle length	12.6	25.2	1.8	81	9.7	19.7	2.4	51
Anal-fin base	15.4	24.4	1.9	81	16.3	22.6	1.4	51
Dorsal-fin length	19.3	31.3	2.1	81	19.2	25.8	1.4	51
Pelvic-fin length	11.4	27.3	2.2	81	11.6	16.8	1.2	51
Pectoral-fin length	16.5	26.6	1.8	81	13.4	23.6	2.4	51
Head length	20.7	33.0	2.1	81	17.4	33.0	2.4	51

The QGIS software (QGIS Development Team, 2025) was used to prepare distribution maps (Figure 1), illustrating the new records of *Astyanax courensis* and *Psalidodon goyanensis*. These specimens originate from several locations, including Brasília National Park (PARNA Brasília), the Águas Emendadas Ecological Station (ESECAE), both in the Federal District, and Serra do Tombador, in the municipality of Cavalcante (State of Goiás). Notably, both Brasília National Park (PARNA) and the Águas Emendadas Ecological Station (ESECAE) are officially designated conservation units. The PARNA, located only 10 km from downtown Brasília, is drained by streams belonging to both the Upper Paraná and Upper Tocantins basins. The ESECAE, in turn, contains the headwaters of the Maranhão River, an important tributary of the Upper Tocantins basin. Finally, the Serra do Tombador is a Private Natural Heritage Reserve located approximately 330 km from Brasília and is drained by streams of the Upper Tocantins basin.

RESULTS

New occurrence records were obtained for *Astyanax courensis* and *Psalidodon goyanensis*, all within the Upper Tocantins basin (Figure 1). For *A. courensis*, the new records include Brasília National Park; in the Federal District, the administrative regions of Fercal, Planaltina, Brazlândia, and Sobradinho; and, in the State of Goiás, the municipality of Planaltina. For *P. goyanensis*, the new records include Brasília National Park; in the Federal District, the administrative regions of Planaltina and Sobradinho; and, in the State of Goiás, the municipality of Cavalcante (Table 1).

To distinguish the species, we used the diagnosis of *Astyanax courensis* (Figure 2), which differs from *Psalidodon goyanensis* by having a more elongated and less deep body (body depth 28-34% of standard length); vertically elongated humeral blotch extending across 3-4 scale rows; rounded and diffuse caudal blotch that slightly overlaps the median caudal-fin rays; 35-36 lateral-line scales; 6-7 scale rows dorsal to and 5-6 ventral to the lateral line, and 20-23 anal-fin rays (Bertaco et al., 2010).

On the other hand, *Psalidodon goyanensis* (Figure 3) has a deeper, more laterally compressed body (body depth 36-42% SL); complete lateral line; oval and well-defined humeral blotch, that does not extend beyond the fourth scale row; conspicuous and sharply defined caudal blotch, that clearly extends over the median caudal-fin rays; 35-37 lateral-line scales; 6-7 scale rows dorsal to and 5-6 ventral the lateral line; and 24-28 anal-fin rays (Bertaco et al., 2010; Terán et al., 2020). Additionally, *P. goyanensis* differs by having 2-3 pentacuspid teeth on the inner premaxillary row and

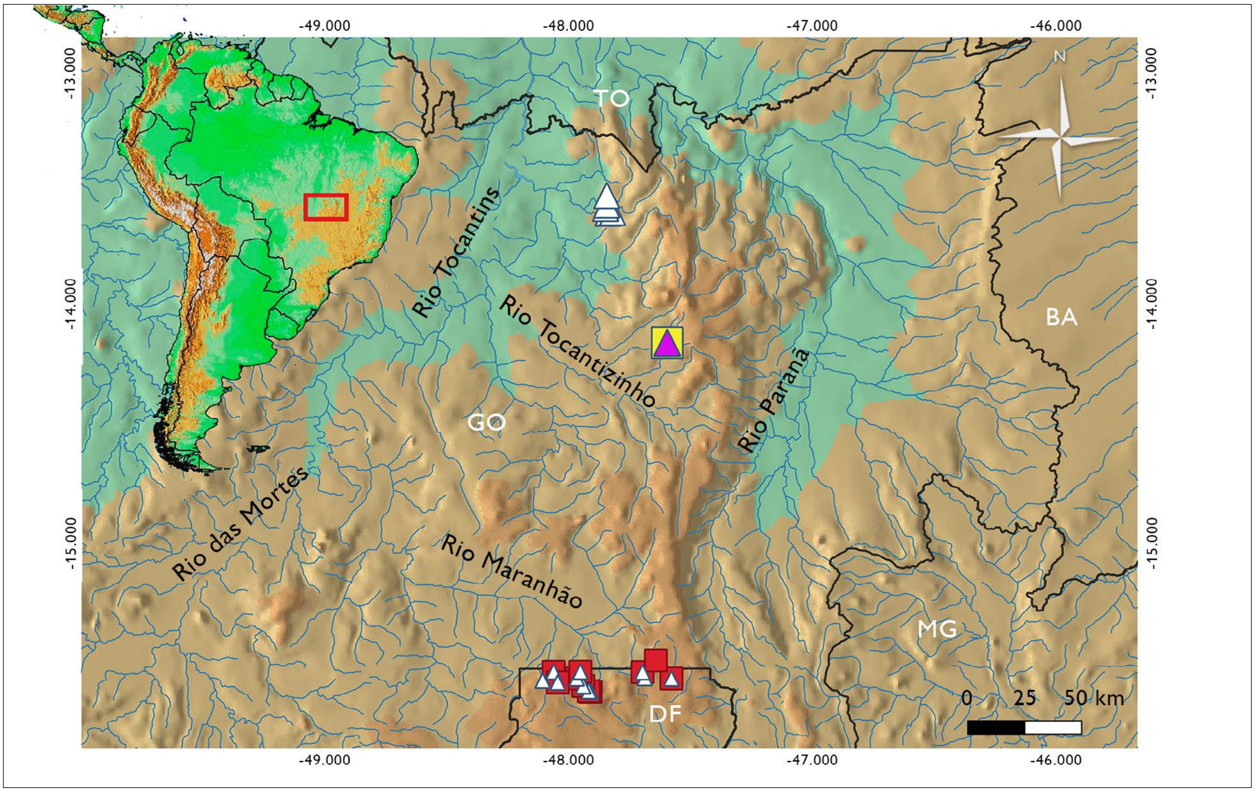

Figure 1. Map of new records of *Astyanax courensis* and *Psalidodon goyanensis* in tributaries of the Federal District and the State of Goiás. Sources: purple triangle = holotype of *Psalidodon goyanensis*; white triangle = new records of *P. goyanensis*; yellow square = holotype of *Astyanax courensis*; red square = new records of *A. courensis*. Map: Firmino (2025).

Figure 2. Left lateral view of *Astyanax courensis*, CIUnB 533, 20.3 mm SL. Image: Firmino (2025).

Figure 3. Left ateral view of *Psalidodon goyanensis*, CIUnB 1358, 21.5 mm SL. Image: Firmino (2025).

4-5 tricuspid teeth on the anterior dentary (Bertaco et al., 2010), whereas *A. courensis* has predominantly tricuspid teeth in both tooth rows (Bertaco et al., 2010).

The type locality of *Astyanax courensis* is the Rio dos Couros basin, Tocantinzinho River, Alto Paraíso de Goiás, Chapada dos Veadeiros, Goiás State (Bertaco et al., 2010). The new records are: Distrito Federal: Brasília National Park, Dois Irmãos Stream; 15,5505° S; 48,1058° W; 29/IX/2010; collector and identifier PPU Aquino; 3, sex indet., CIUnB 533; Brasília National Park, Palma River; 15,51487° S; 48,06089° W; 29/IX/2010; collector and identifier PPU Aquino; 3, sex indet., CIUnB 513; Fercal, Buraco Stream; 15,59683° S; 47,90883° W; 28/IX/2010; collector and identifier PPU Aquino; 5, sex indet., CIUnB 541; Fercal, Maranhão River, Pindaíba Stream; 15,594303° S; 47,91675° W; 28/I/2006; collector and identifier PPU Aquino; 17, sex indet., CIUnB 139; Fercal, Taquari Stream; 15,57313° S; 47,93952° W; 11/V/2011; collector and identifier PPU Aquino; 61, sex indet., CIUnB 868; Fercal, Cafuringa Stream; 15,54164° S; 47,96622° W; 28/IX/2010; collector and identifier PPU Aquino; 5, sex indet., CIUnB 555; Planaltina, Águas Emendadas Ecological Station, Vereda Grande Stream; 15,54216° S; 47,57801° W; 8/IV/2017; collector and identifier PPU Aquino; 80, sex indet., CIUnB 837; Planaltina, Brejo Stream; 15,540183° S; 47687433° W; 24/VII/2023; collector A. Firmino, R. Nayara; identifier R. Nayara; 39, sex indet., CIUnB 2267; Planaltina, Maranhão River, downstream dam; 15,516128° S, 47,696348° W; 23/VII/2023; collector A. Firmino, R. Nayara; identifier R. Nayara; 41, sex indet., CIUnB 2312; Brazlândia, Poço Azul; 15,558° S; 48,044° W; 4/VI/2023; collector A. Firmino, R. Nayara; identifier R. Nayara; 12, sex indet., CIUnB 1941; Sobradinho, Salinas Morro da Pedreira Stream; 15,514408° S; 47,951416° W; 9/VI/2015; collector and identifier PPU Aquino; 1, sex indet., CIUnB 1450. Goiás: Planaltina, Contendas Stream; 15,46805° S; 47,64161° W; 19/IX/2014; collector and identifier PPU Aquino; 2, sex indet., CIUnB 1051.

The type locality of *Psalidodon goyanensis* is the Rio dos Couros basin, Tocantinzinho River, Alto Paraíso de Goiás, Chapada dos Veadeiros, Goiás State (Bertaco et al., 2010). The new records are: Distrito Federal: Brasília National Park, Buraco Stream; 15,59885° S; 47,896853° W; 28/I/2006; collector and identifier PPU Aquino; 16, sex indet., CIUnB 128; Planaltina, Águas Emendadas Ecological Station, Vereda Grande Stream; 15,52037° S; 47,56781° W; 8/IV/2011; collector and identifier PPU Aquino; 74, sex indet., CIUnB 840; Sobradinho, Ouro Stream; 15,512° S; 47,930° W; 8/VI/2023; collector and identifier A. Firmino; 6, sex indet.; CIUnB 1768. Goiás: Cavalcante, Serra do Tombador, Conceição River; 13,63500° S; 47,83826° W; 19/XI/2016; collector and identifier PPU Aquino; 5, sex indet., CIUnB 1375; Cavalcante, Serra do Tombador, Mirante Stream; 13,63241° S; 47,81905° W; 18/XI/2016; collector and identifier PPU Aquino; 14, sex indet., CIUnB 1355; Cavalcante, Serra do Tombador, Santa Rita River; 13,61496° S; 47,84793° W; 18/XI/2016; collector and identifier PPU Aquino; 19, sex indet., CIUnB 1362; Cavalcante, Serra do Tombador, Santa Rita River; 13,60140° S; 47,84300° W; 18/XI/2016; collector and identifier PPU Aquino; 10, sex indet., CIUnB 1358; Cavalcante, São Félix River; 13,562679° S; 47,715224° W; 13/X/2019; collector and identifier PPU Aquino; 4, sex indet., CIUnB 1503.

DISCUSSION

Acestrorhamphidae is currently considered one of the most diverse families within the order Characiformes (Melo et al., 2024), and it presents numerous challenges for the taxonomic delimitation and systematics of its taxa. The species *Astyanax courensis* and *Psalidodon goyanensis* are emblematic examples of these taxonomic challenges (e.g., Bertaco et al., 2010; Terán et al., 2020). Both were originally assigned to the genus *Astyanax* and included in the *Astyanax scabripinnis* complex due to their general morphological similarity, such as body shape and the presence of a characteristic humeral blotch (Bertaco et al., 2010).

However, recent studies have indicated that *Astyanax*, as delimited based on the works of Eigenmann (1917, 1921), does not correspond to a natural group. As a result, other genera have been resurrected (such as *Psalidodon*, following Terán et al., 2020) or newly described (such as *Makunaima* Terán et al., 2020) to accommodate the diversity previously assigned to *Astyanax*.

The taxonomic advances reinforce the importance of updated and properly documented records in scientific collections, such as those presented in this study. Consequently, these findings expand our understanding of the spatial distribution of the species reported herein, contributing to the reconstruction of the history of Neotropical biodiversity, particularly in the Tocantins-Araguaia basin. Much of the knowledge about Neotropical fishes comes from ichthyological collections (Bogotá-Gregory et al., 2020), which serve as essential tools for studying biodiversity through time.

The Upper Tocantins river basin deserves attention due to its ecological importance and the number of associated endemic species (Campos et al., 2025). Unfortunately, portions of the Upper Tocantins basin have been affected by the expansion of agricultural activities directed toward grain production, such as corn and soy (Barbosa et al., 2017). In addition, cattle ranching is practiced on properties with large territorial extensions (Klink & Machado, 2005; Barbosa et al., 2017). This alteration of the natural environment leads to the replacement of *Cerrado* vegetation by monocultures, which in turn directly impacts negatively the existing water bodies (Sano et al., 2007).

Although some of the new records of *A. courensis* and *P. goyanensis* are located within strict-protection conservation units, such as Brasília National Park and the Águas Emendadas Ecological Station, others are not protected by any legal conservation designation. Notably, ecotourism, as observed in Brasília National Park, exerts negative impacts on the local fauna and flora (Berto & Luquez, 2016). These impacts arise from factors such as intense vehicle traffic, significant waste generation, and the improper use of water resources (Berto & Luquez, 2016). It is important that the impacts on the species studied here continue to be monitored; however, the expansion of their known distribution, including in the aforementioned conservation units, supports their classification as Least Concern (Melo et al., 2024).

CONCLUSION

Overall, the distribution of *Astyanax courensis* and *Psalidodon goyanensis* shows their notable presence in the Upper Tocantins river basin. Studying and mapping their distribution is essential for several purposes, including understanding population variation, conducting biogeographic studies, expanding knowledge on evolutionary processes, and identifying the threats faced by these species (e.g., Lomolino et al., 2010; Primack, 2010).

Additionally, these data may contribute to phylogenetic studies that significantly assist in more accurately delimiting species complexes of the genus *Astyanax* in the headwaters of the Central *Cerrado* (Castro et al., 2014). Such studies are essential to clarify if other records correspond to already described species, or whether regional variations indicate the presence of new species that have not yet been formally described (Lima et al., 2003; Lomolino et al., 2010).

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AUTHORS' CONTRIBUTION

R. N. S. Oliveira contributed to project administration, formal analysis, conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review, and editing); A. Firmino contributed to formal analysis, data curation, investigation, and methodology; and V. B. Slobodian contributed to funding acquisition, conceptualization, resources, supervision, validation, and writing (review and editing).

New record of *Lepidodactylus lugubris* (Squamata: Gekkonidae) from Amazonas, Brazil, and notes on its feeding biology in urbanized environment

Novo registro de *Lepidodactylus lugubris* (Squamata: Gekkonidae) para o Amazonas, Brasil, e notas sobre sua biologia alimentar em ambiente urbanizado

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Abstract: We report the first documented record of the mourning gecko, *Lepidodactylus lugubris*, from the state of Amazonas, Brazil, expanding its known distribution within the Brazilian Amazon. Additionally, we provide observations on the feeding behavior of this species in urban environments of Belém, Pará. Individuals were frequently observed feeding on the nectar of various plant species, as well as on sugary artificial foods. These observations confirm the species' dietary plasticity and its ability to exploit anthropogenic food sources, which may facilitate its establishment and persistence in urbanized areas.

Keywords: Brazilian Amazon. Common smooth-scaled gecko. Gekkota. Mourning gecko.

Resumo: Relatamos o primeiro registro documentado da lagartixa *Lepidodactylus lugubris* no estado do Amazonas, Brasil, ampliando sua distribuição conhecida na Amazônia brasileira. Além disso, apresentamos observações sobre o comportamento alimentar dessa espécie em ambientes urbanos de Belém, Pará. Indivíduos foram frequentemente observados alimentando-se do néctar de diversas espécies de plantas, bem como de alimentos artificiais açucarados. Essas observações confirmam a plasticidade alimentar da espécie e sua capacidade de explorar fontes de alimento de origem antrópica, o que pode facilitar seu estabelecimento e persistência em áreas urbanizadas.

Palavras-chave: Amazônia brasileira. Lagartixa-de-parede. Gekkota. Osga.

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INTRODUCTION

Gekkonidae is the largest family of geckos, comprising about 60 genera and 1,734 species distributed in all biogeographic regions, but they are most numerous in tropical regions (Han et al., 2004; Gamble et al., 2008, 2015; Midtgaard, 2025). Until now, three species of Gekkonidae have been recorded from the Brazilian Amazon, but two of them, *Hemidactylus mabouia* (Moreau de Jonnès, 1818) and *Lepidodactylus lugubris* (Duméril & Bibron, 1836), are introduced species from the Old World (Ávila-Pires, 1995; Hoogmoed & Ávila-Pires, 2015; D'Angiolella et al., 2021). The only native species currently known from the region is *Hemidactylus palaichthus* Kluge, 1969 (Ávila-Pires, 1995). To date, no studies have provided evidence that these two invasive gecko species are directly responsible for declines in native species in Brazil.

The mourning gecko (*L. lugubris*) is native to coastal regions of the Indian and Pacific Oceans, where it is widely distributed (Hoogmoed & Ávila-Pires, 2015). It is a small, predominantly parthenogenetic gecko whose females reproduce clonally, producing genetically identical offspring without the need for males (Radtkey et al., 1995; Arteaga et al., 2022). This reproductive strategy enables single individuals to establish new populations with remarkable ease (Radtkey et al., 1995). This species is nocturnal and highly adaptable, occupying a broad range of habitats including coastal forests, urban areas, and human dwellings (Liebgold et al., 2019; Arteaga et al., 2022). Its diet is equally flexible, consisting of small arthropods, nectar, and occasionally artificial sugary food sources (Arteaga et al., 2022; Palheta et al., 2025).

These biological traits—parthenogenesis, dietary plasticity, behavioral flexibility, and tolerance to disturbed environments—make *L. lugubris* one of the most successful invasive reptiles worldwide. As a result, it has independently colonized multiple countries across the Americas, from Florida to northern Brazil, as well as several Caribbean islands, primarily via maritime cargo transport and possibly also by air transport (Hoogmoed & Ávila-Pires, 2015; Behm et al., 2019; Nania et al., 2020; Urra et al., 2020). Despite its wide distribution, this species was only recently recorded in Brazil, in the Brazilian Amazon (state of Pará) and the Atlantic Forest (state of Bahia) (Hoogmoed & Ávila-Pires, 2015; D'Angiolella et al., 2021; Bandeira & Missassi, 2022). The mourning gecko is found mainly in gardens, backyards and inside of residences in urban environments (Hoogmoed & Ávila-Pires, 2015), but there is little information about the feeding behavior of this species in these environments (D'Angiolella et al., 2021; Palheta et al., 2025). Given this knowledge gap, our study aims to document feeding events of *L. lugubris* in an urban area of the Brazilian Amazon, characterize the types of food items consumed, and evaluate the behavioral contexts in which these events occur.

MATERIAL AND METHODS

The specimens reported in this paper were observed in two localities of the Brazilian Amazon: 1) The research *Campus* of the *Instituto Nacional de Pesquisas da Amazônia* (INPA), an urban area of the city of Manaus, state of Amazonas, and 2) five places in the city of Belém, state of Pará (Table 1). Belém and Manaus are situated in the eastern and central sectors of the Brazilian Amazon Basin, respectively, and both exhibit a humid equatorial climate (Af, Köppen classification) characterized by high precipitation, persistently elevated humidity, and minimal thermal seasonality (Peel et al., 2007).

The specimen from INPA was not collected, but it was photographed with an Olympus Tough T-4 camera. The specimens from Belém were photographed with a Canon 70D with a 100 mm f/2.8 macro lens and a Speedlite 600ex flash, as well as with mobile phone cameras, and no individuals were collected. The observations were made opportunistically by the authors and two additional observers between 2017 and 2023. Since the specimens were not marked and the observations were made sporadically, it is not possible to determine whether the records from the same locality correspond to a single individual or to multiple ones.

Table 1. Locations, dates, and diel periods of feeding events of *Lepidodactylus lugubris* documented in urban environments of Belém City, state of Pará, Brazil.

Feeding behaviour	Locations	Coordenates	Date	Diel period	Figure
Feeding on nectar of ambarella flower (*Spondias dulcis*)	Third floor veranda of residence A	1° 25' 32.0" S 48° 29' 13.8" W	31/V/2017	Night	1C
Feeding on nectar of jungle geranium flowers (*Ixora coccinea*)	Garden of residence B	1° 25' 51.1" S 48° 27' 11.7" W	13/V/2020	Dusk	1D
Feeding on extrafloral nectaries on the stem of an American sicklepod (*Senna obtusifolia*)	Garden of residence B	1° 25' 51.1" S 48° 27' 11.7" W	12/XII/2020	Dusk	1E
Feeding on nectar of milkvine flower (*Matelea denticulata*)	Garden of residence B	1° 25' 51.1" S 48° 27' 11.7" W	17/VII/2023	Dusk	1H
Feeding on extrafloral nectaries on the pitcher of tropical pitcher plant (*Nepenthes* sp.)	Garden of residence C	1° 21' 58.6" S 48° 26' 43.9" W	04/II/2024	Night	1F
Feeding on extrafloral nectaries on a branch of an inga tree (*Inga* sp.)	Garden of residence C	1° 21' 58.6" S 48° 26' 43.9" W	28/VI/2025	Night	1G
Feeding on cake crumbs	Inside residence A	1° 25' 32.0" S 48° 29' 13.8" W	22/IX/2018	Night	2A
Feeding on chocolate milk	Inside residence A	1° 25' 32.0" S 48° 29' 13.8" W	2017	Night	-
Feeding on sweetened coffee	Inside residence B	1° 25' 51.1" S 48° 27' 11.7" W	2020	Night	-
Feeding on banana	Inside residence B	1° 25' 51.1" S 48° 27' 11.7" W	20/X/2020	Day	2F
Feeding on fruit juice inside a glass cup	Inside residence D	1° 26' 53.6" S 48° 27' 32.9" W	2018	Night	2B
Feeding on honey	Inside residence D	1° 26' 53.6" S 48° 27' 32.9" W	2018	Night	2G
Feeding on grape jelly	Inside residence D	1° 26' 53.6" S 48° 27' 32.9" W	2018	Night	-
Feeding on jaboticaba wine	Inside residence E	1° 26' 1.50" S 48° 27' 23.3" W	04/XI/2020	Night	2D-2E
Feeding on grape wine	Inside residence E	1° 26' 1.50" S 48° 27' 23.3" W	2020	Night	-
Feeding on beer	Inside residence E	1° 26' 1.50" S 48° 27' 23.3" W	2020	Night	-
Feeding on melted ice cream	Waterfront complexes (Estação das Docas)	1° 26' 53.5" S 48° 30' 0.28" W	11/IV/2020	Night	2C
Licking the feces of a palm tanager (*Thraupis palmarum*)	Outdoor area of residence B	1° 25' 51.1" S 48° 27' 11.7" W	23/XI/2020	Night	-

Figure 1. A-B) New record of *Lepidodactylus lugubris* from state of Amazonas, Brazil; C-H) New record of feeding events of *Lepidodactylus lugubris* on floral and extrafloral nectar sources in urban environments in the Belém City, Pará, Brazil: C) feeding on nectar of ambarella (*Spondias dulcis*) on the third floor veranda of a residence at night; D) feeding on nectar of jungle geranium flowers (*Ixora coccinea*) in a garden at night; E) feeding on extrafloral nectaries on the stem of an American sicklepod (*Senna obtusifolia*) in a garden at dusk; F) feeding on extrafloral nectaries on the pitcher of a tropical pitcher plant (*Nepenthes*) in a garden at night; G) feeding on extrafloral nectaries on a branch of an inga tree (*Inga* sp.) in a garden at night; H) feeding on nectar of a milkvine flower (*Matelea denticulata*) in a garden at dusk. Photos: C. A. Perez (A, B), C. A. C. Favacho (C, F, G), F. S. Carvalho-Filho (D, E, H).

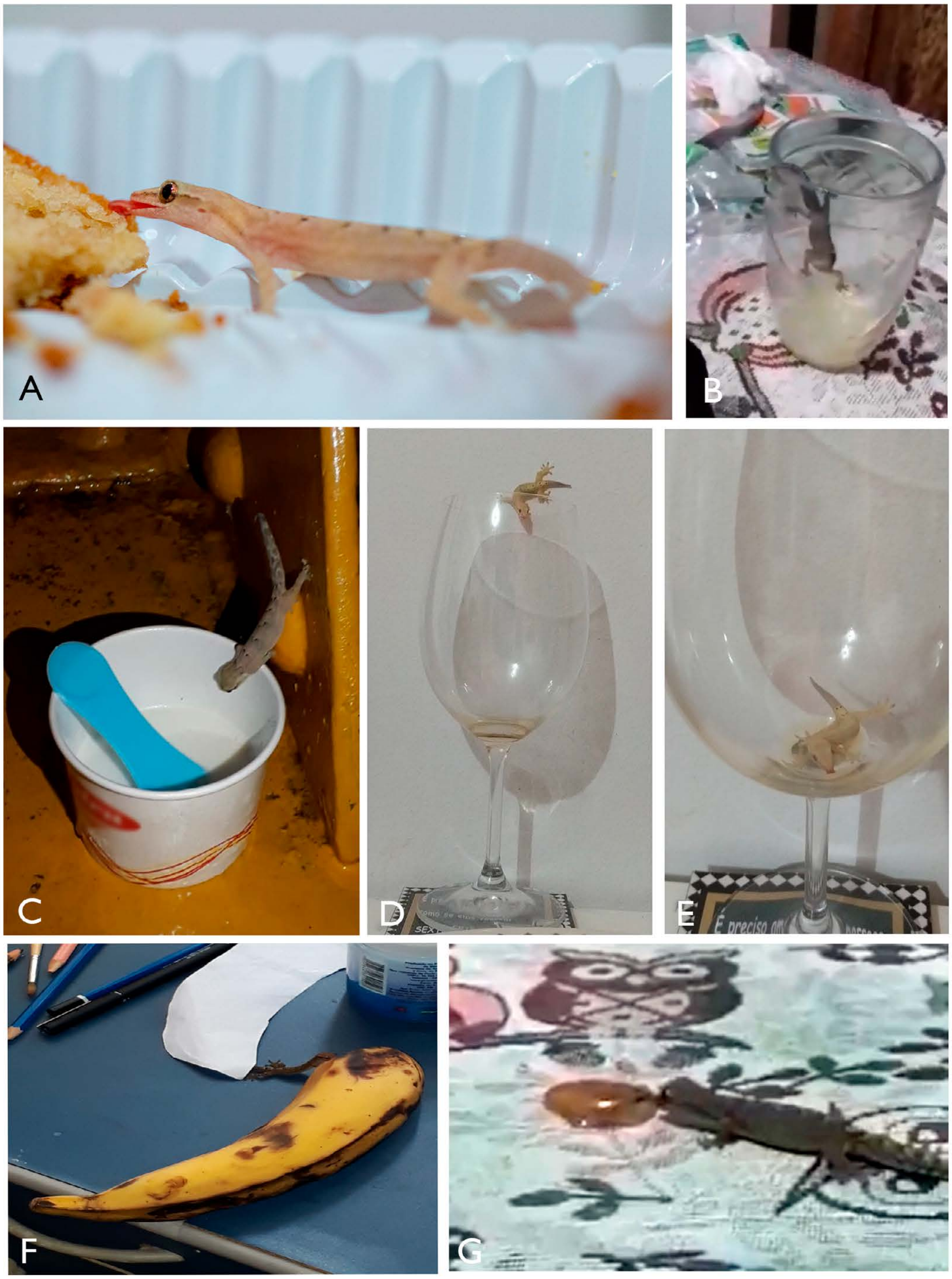

Figure 2. Feeding events of *Lepidodactylus lugubris* on artificial and natural food sources in urban environments in Belém City, state of Pará, Brazil: A) feeding on cake crumbs; B) feeding on fruit juice inside a glass cup; C) feeding on melted ice cream; D-E) feeding on jaboticaba wine; F) feeding on banana; G) feeding on honey. Photos: C. A. C. Favacho (A), F. S. Carvalho-Filho (C, F), J. H. Viana (D, E), L. J. Guerreiro (B, G).

RESULTS AND DISCUSSION

At INPA, one specimen of mourning gecko was found under tree bark during the daytime, about 08:30 a.m., in front of a building in an area with sparse vegetation on 28th April 2017 (Figures 1A-1B). It was shedding its skin when it was found (Figure 1B). This is the first documented record of this species in the state of Amazonas and the second record from the Brazilian Amazon, where it had been recorded only in the state of Pará (Hoogmoed & Ávila-Pires, 2015; D'Angiolella et al., 2021; Palheta et al., 2025). Manaus is located about 1,294 km from Belém, if following a straight line, and the aerial and fluvial trade between these cities is intense. This may have favored the dispersal of the mourning gecko, as this species is primarily spread through transport on boats (Nania et al., 2020). However, considering that Manaus is one of the main commercial centers of the Brazilian Amazon region, receiving cargo from many parts of the world, it is possible that the population of *L. lugubris* from Manaus may have been established independently from that of Pará.

Specimens from Belém were observed six times feeding on nectar of native and exotic plants at dusk and during the night in 2017 and 2025 (Table 1). One individual was observed feeding on the nectar of the exotic ambarella flower, *Spondias dulcis* (Figure 1C), located in a vase on the third floor veranda of a residence. Other specimens were observed licking nectar from milkvine flowers, *Matelea denticulata* (Figure 1H), from extrafloral nectaries on the stem of American sicklepod, *Senna obtusifolia* (Figure 1E), on a branch of an inga tree, *Inga* (Figure 1G), an exposed nectary (a flower with a dropped corolla tube) of exotic jungle geranium, *Ixora coccinea* (Figure 1D) and from the exotic pitcher of a tropical pitcher plant, *Nepenthes* (Figure 1F), all of which were in gardens (Table 1).

Geckos are essentially carnivorous, feeding mainly on invertebrates (predominately insects and arachnids) (Bauer, 2013). However, some species also feed on sugary substances such as the nectar of flowers (Taylor & Gardner, 2014) and honeydew produced by insects (Fölling et al., 2001), including the mourning gecko, that has been previously observed feeding on nectar (Perry & Ritter, 1999; Nafus, 2012; Palheta et al., 2025). Like the mourning gecko, ambarella is a species from Asia that has been introduced in many parts of the world (Mitchell & Daly, 2015), and it is found in gardens, plantations, and secondary forests. Both ambarella and the mourning gecko are found together in some localities along their distribution; thus, this plant may be a source of nectar for mourning geckos in Brazil and in other parts of the world where they co-occur. The species of the genus *Spondias* are pollinated by Hymenoptera, mainly bees (Roubik et al., 1986; Nadia et al., 2007), but it is possible that the mourning gecko is a secondary pollinator, as suggested by studies on other plant species (Perry & Ritter, 1999; Nafus, 2012). Several lizard species have been documented acting as occasional or even effective pollinators, including multiple geckos, which visit flowers to feed on nectar and inadvertently transfer pollen (Olesen & Valido, 2003; Romero-Egea et al., 2023). This broader ecological pattern strengthens the hypothesis that *L. lugubris* may also contribute to the pollination of *Spondias* species when nectar foraging occurs.

In the city of Belém, *L. lugubris* have also been observed feeding on grape jelly, jaboticaba and grape wines (Figures 2D-2E), beer, sweetened coffee, chocolate milk, melted ice cream (Figure 2C), artificial and natural fruit juices (Figure 2B), industrialized honey (Figure 2G), and cake crumbs (Figure 2A) in residences during the night under artificial light. Arteaga et al. (2022) and Palheta et al. (2025) likewise reported a comparable feeding behavior for this species, although they did not provide photographic records. To reach some of these food items, the specimens were observed entering cups, bottles, and climbing dirty dishes inside of sinks, even in the presence of humans in illuminated environments (Figure 2).

In addition to sugary artificial products, *L. lugubris* was also observed feeding on fruits in the urban area of Belém. Some specimens have been observed feeding on partially peeled bananas and pineapples during the daytime and at night. On one of these occasions, the first author observed a mourning gecko walking on the floor, for about two meters, to reach a banana placed close to it on a work table at 10:00 am (Figure 2F). The mourning gecko did not appear intimidated by the presence of the observer; it climbed up the table leg and reached the banana. This observation suggests that mourning geckos, like other species of Gekkonidae, may possess well-developed nasal chemosensory abilities, as the individual was able to locate a banana located at least two meters away (Schwenk, 1993). One specimen was also observed licking the feces of a palm tanager (*Thraupis palmarum* (Wied, 1821)) on a glass window during the night. It is possible the specimen was attracted to the feces since they contained fragments of undigested papaya pulp.

As observed in this study and in others already published (Short & Petren, 2008; Sakai, 2019), *L. lugubris* shows low aversion to human presence, and this behavior may be linked to the species' broad distribution as animal–human interactions become increasingly close. Indeed, *L. lugubris* is often described as a 'human-commensal' gecko, thriving in synanthropic environments and exploiting anthropogenic habitats (Carranza & Arnold, 2006; Behm et al., 2019). This pattern of commensalism and urban-habitat colonization is well documented also for the invasive tropical house-gecko *H. mabouia* (Bonfiglio et al., 2006; Sousa et al., 2017). Given the clear ecological parallel between *L. lugubris* and *H. mabouia*, similar future scenarios can be suggested for the studied species, as human-dominated landscapes expand and human–animal contact intensifies, *L. lugubris* may broaden its geographical range further, especially in human-dominated areas.

The availability of sugary food resources in urban environments may play a key role in facilitating the establishment of *L. lugubris* in human-dominated areas. Food waste and the presence of sweet residues in kitchens, garbage sites, and commercial areas provide abundant and predictable resources for foraging. Combined with the species' low aversion to human presence, these conditions may significantly enhance its ability to occupy and persist within buildings. As *L. lugubris* readily exploits anthropogenic food sources and tolerates frequent proximity to humans, its expansion in urban structures is likely to intensify, reinforcing its status as a successful invasive species in anthropogenic habitats.

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F. S. Carvalho Filho contributed to conceptualization, data curation, investigation, methodology and writing (original draft, review and editing); and C. A. C. Favacho contributed to conceptualization, investigation, methodology and writing (review and editing).

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New localities of *Myrmecophaga tridactyla* (Linnaeus, 1758), Giant Anteater (Myrmecophagidae), in the southern region of Minas Gerais state, Brazil

Novas localidades de *Myrmecophaga tridactyla* (Linnaeus, 1758), tamanduá-bandeira (Myrmecophagidae), na região sul do estado de Minas Gerais, Brasil

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Abstract: *Myrmecophaga tridactyla* (Linnaeus, 1758), species commonly known as the giant anteater, has had its geographic distribution reduced due to several factors (natural and anthropogenic environmental changes, low reproductive rate, resource depletion, conflicts and hunting). The species is currently classified as 'Vulnerable' at local, national and global levels. This study reports two new records of *M. tridactyla* in southern Minas Gerais, Brazil, in the municipalities of Lavras and Santo Antônio do Amparo, with a six-month interval between sightings. These records, obtained through direct observation and camera traps, identify new occurrence locations for the species and reinforce the need for further studies and targeted conservation initiatives.

Keywords: Endangered species. Mammal. Xenarthra. Geographic distribution.

Resumo: *Myrmecophaga tridactyla* (Linnaeus, 1758), espécie comumente conhecida como tamanduá-bandeira, teve sua distribuição geográfica reduzida devido a diversos fatores (alterações ambientais naturais e antropogênicas, baixa taxa reprodutiva, esgotamento de recursos, conflitos e caça). A espécie está atualmente classificada como 'vulnerável' em níveis local, nacional e global. Este estudo relata dois novos registros de *M. tridactyla* no sul de Minas Gerais, Brasil, nos municípios de Lavras e Santo Antônio do Amparo, com um intervalo de seis meses entre os avistamentos. Esses registros, obtidos por meio de observação direta e armadilhas fotográficas, identificam novas ocorrências da espécie e reforçam a necessidade de mais estudos e iniciativas de conservação direcionadas.

Palavras-chave: Espécie ameaçada de extinção. Mamífero. Xenarthra. Distribuição geográfica.

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INTRODUCTION

The Giant Anteater (*Myrmecophaga tridactyla*, Linnaeus, 1758) is a species that, along with the genus *Tamandua* (Linnaeus, 1758), forms the family Myrmecophagidae, primarily characterized by the absence of teeth. The species originally had a distinct geographic distribution from its current one, ranging from southern Belize and Guatemala to northern Argentina (Wetzel, 1982, 1985). It was also found across all six biomes in Brazil: Amazon, *Cerrado*, *Caatinga*, *Pantanal*, *Pampas* (IBGE, 2019).

Due to the ongoing reduction of its natural habitat, *M. tridactyla* is now listed as an endangered species in the 'Vulnerable' category at regional (Minas Gerais state, Brazil; COPAM, 2010), national (Brazil; MMA, 2022), and global scales (Miranda et al., 2025). Surveys conducted by ICMBio indicate probable extinction of the species in the *Pampas*, near extinction in the Atlantic Forest, and a drastic population decline in the Cerrado, being the region with the lowest genetic diversity (Miranda et al., 2022 and references therein). Although studies on mammal fauna are regularly conducted in Minas Gerais (Eduardo & Passamani, 2009; Silva & Passamani, 2009; Machado et al., 2015, 2016, 2017), there were no sightings in the southern part of Minas Gerais until 2016, with the first record being in the Serra da Boa Esperança State Park (PESBE) (Moura et al., 2017).

Recent surveys have recorded the species in two new locations: Lavras and Santo Antônio do Amparo, both municipalities located in southern Minas Gerais. No other records of *M. tridactyla* had been documented in southern Minas Gerais since 2016 until now. This manuscript aims to present these two new locations of the species in southern Minas Gerais, aiming to contribute to future studies related to the conservation biology and protection of the species.

MATERIALS AND METHODS

The records were made in the municipalities of Santo Antônio do Amparo (20° 56' 45" S, 44° 55' 08" W) and Lavras (21° 14' 45" S, 44° 59' 59" W) in the southern part of the state of Minas Gerais, southeastern Brazil (Figure 1). These municipalities are located in an ecotonal region between the Atlantic Forest and *Cerrado* biomes, with the landscape composed of semi-deciduous seasonal forest fragments, riparian forests, cloud forests, *Cerrado sensu stricto*, high-altitude grasslands, and rocky fields (Dalanesi et al., 2004; Scolforo & Carvalho, 2006). The climate classification of the southern region of the state of Minas Gerais is predominantly a humid subtropical climate influenced by monsoons (Cwa), characterized by dry, cold winters and hot, humid summers. In higher-altitude areas, the climate shifts to a highland subtropical climate (Cwb), with dry winters and mild summers, according to the Köppen climate classification (Alvares et al., 2013). The records were obtained during fauna studies using camera traps and direct observation.

RESULTS

On November 18, 2023, an individual of the species was captured on a camera trap at one of the study sites on Fazenda da Lagoa, a property of the Federal University of Lavras (UFLA), in Santo Antônio do Amparo, Minas Gerais. Additionally, as part of the same study, a visual record of a specimen of *M. tridactyla* was made on June 15, 2024, at the Lavras Parque I Condominium in the municipality of Lavras, Minas Gerais (Figure 1). The sighting was reported by the condominium's security guard during a daytime patrol around 9:30 am, and the species was confirmed by one of the authors. The sex of the individual was not identified.

DISCUSSION

The southern region of Minas Gerais is well studied in terms of mammalogy (Eduardo & Passamani, 2009; Silva & Passamani, 2009; Machado et al., 2015, 2016, 2017). However, to date, there has been only one recorded sighting of *Myrmecophaga tridactyla* in the area (Moura et al., 2017). Recent records obtained through camera traps (Figure 2) and visual sightings indicate two new localities in southern Minas Gerais.

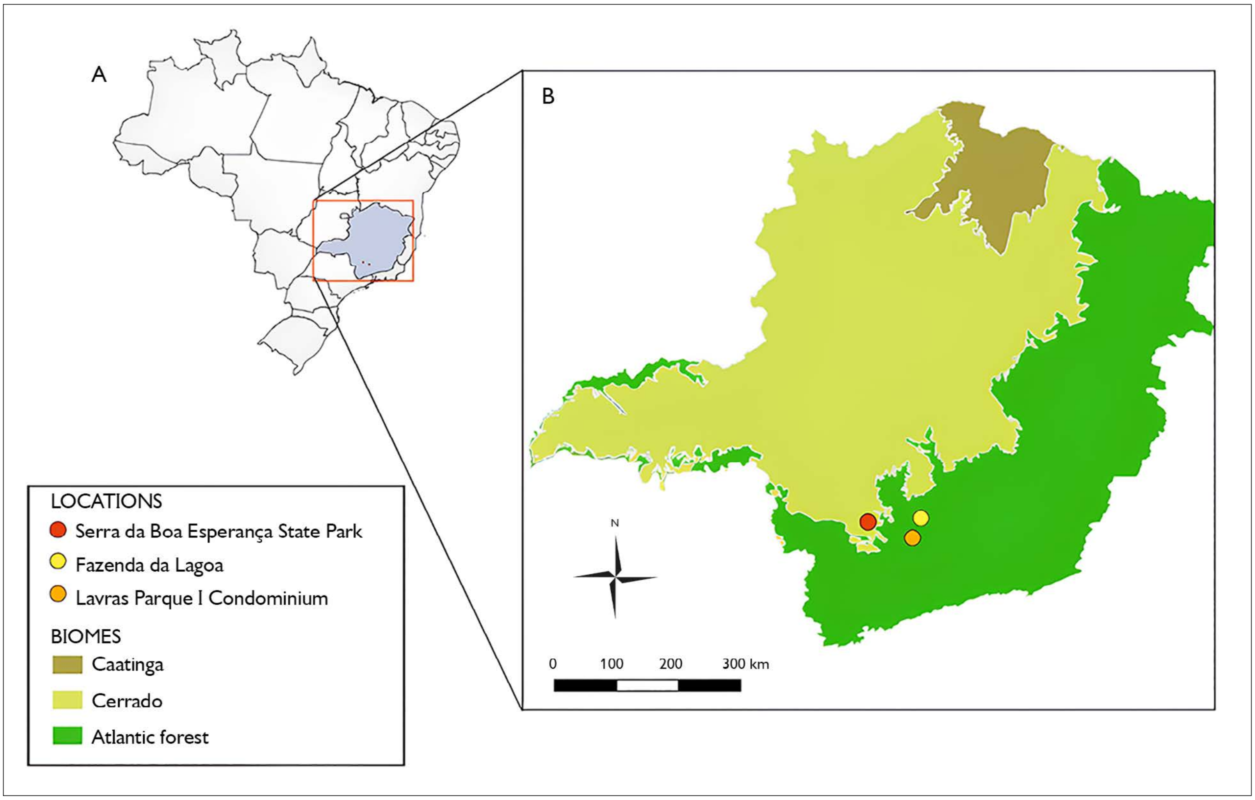

Figure 1. Map showing the locations of *Myrmecophaga tridactyla* records in the southern region of Minas Gerais. Map: Dunia Lasmar (2025).

Figure 2. *Myrmecophaga tridactyla* captured through a camera trap at Fazenda da Lagoa (UFLA), Santo Antônio do Amparo, Minas Gerais. Photo: Aloysio Souza de Moura (2025).

These two new records occurred in a period of only six months and, as the crow flies, are less than 100 km from the Serra da Boa Esperança State Park, where the first sighting of *M. tridactyla* was reported in southern Minas Gerais (Moura et al., 2017). Both locations are separated by 39 km. The proximity of the locations and the similarity of the landscape (*Cerrado*/Atlantic Forest ecotone) may have been factors that favored the appearance of the individual and the records. As discussed by Miranda et al. (2022), the species appears to have historically expanded its demographic distribution in the *Cerrado* and *Pantanal*, proving to be more adapted to the conditions of these biomes.

Previous studies indicate that the species is threatened mainly by habitat loss due to the road network, agriculture, and fires, among other factors (Miranda et al., 2015). *Myrmecophaga tridactyla* is currently regionally extinct in several parts of southeastern Brazil, such as Espírito Santo (Passamani & Mendes, 2007) and possibly Rio de Janeiro (Bergallo et al., 2000), making it dependent on protected areas for its survival, particularly in anthropogenic landscapes (Versiani et al., 2021).

The spatial proximity of the observed records suggests a potential population expansion of the species in the region, which may be directly related to the environmental conditions found in this ecotonal habitat, which resembles locations with high population densities of *M. tridactyla*, such as Serra da Canastra National Park (Shaw et al., 1985). Recent studies indicate an adaptation of the species to fragmented and modified landscapes (Santos et al., 2019; Miranda et al., 2022), demonstrating its resilience and possible exploration of new areas.

In this context, identifying and reporting new localities where *M. tridactyla* is present becomes even more relevant. These records provide crucial information that can be used to guide and support formulating public policies and conservation strategies. With a more detailed understanding of the species' distribution patterns, it is possible to make more assertive and effective decisions to protect habitats, especially in regions where the species shows signs of expansion. Thus, conservation of these environments can be better achieved, ensuring the long-term sustainability of the species population in the area.

This study presented two new localities for *Myrmecophaga tridactyla*, in the south of Minas Gerais state, southeastern Brazil. Given the current conservation status of the species, new records in regions not previously observed are extremely important for understanding its distribution and adaptation to environmental disturbances. Thus, these data can assist in future studies.

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AUTHORS' CONTRIBUTION

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New occurrence of sirenians in the Pirabas Formation (Miocene), Pará, Brazil

Nova ocorrência de sirênios na Formação Pirabas (Mioceno), Pará, Brasil

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Abstract: This note reports a new occurrence of sirenian fossils in the Pirabas Formation, at the Praia de Fortalezinha site, in the Maracanã municipality, Pará, Brazil. The aim of this preliminary study is to corroborate the palaeobiogeography knowledge of sirenians from the Miocene of Eastern Amazon. Unlike most previous records, which consists mainly of vertebrae and ribs, the material collected from a sedimentary block chacterised as *packstone/grainstone* contains ribs and cranial parts, including a fragmented rostrum (premaxilla with incisor tooth remains), key elements for an accurate taxonomic identification. This finding broadens the fossil record of Sirenia within the Pirabas Formation. The cranial elements will enable a more detailed taxonomic identification and provide relevant data on the distribution and evolution of sirenians in South America during the Neogene.

Keywords: Sirenia. New record. Palaeobiogeography. Eastern Amazon.

Resumo: A presente nota trata de uma nova ocorrência de fósseis de sirênios na Formação Pirabas, localizada na Praia de Fortalezinha, município de Maracanã, Pará, Brasil. O objetivo deste estudo preliminar é contribuir para o conhecimento paleobiogeográfico dos sirênios no Mioceno da Amazônia oriental. Diferente da maioria dos registros anteriores, que consistem principalmente de vértebras e costelas, o material, coletado em um bloco sedimentar caracterizado como *packstone/grainstone*, contém costelas e partes cranianas, incluindo rostro (pré-maxilar com restos de dente incisivo) fragmentado, elementos-chave para uma identificação taxonômica precisa. O novo achado amplia o registro fossilífero dos sirênios na Formação Pirabas. Os elementos cranianos encontrados irão possibilitar uma identificação taxonômica mais detalhada e fornecer dados relevantes sobre a distribuição e a evolução dos sirênios na América do Sul durante o Neógeno.

Palavras-chave: Sirenia. Novo registro. Paleobiogeografia. Amazônia oriental.

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INTRODUCTION

The outcrops known as the Pirabas Formation were discovered by Domingos Soares Ferreira Penna (Penna, 1876), while on an expedition in the São João de Pirabas district, at the Primavera municipality, Pará state, Brazil. Subsequently, the formation was documented at various locations in Pará, where it is most extensive, as well as in the Maranhão and Piauí states. Due to this large extension, Távora et al. (2010) compiled information about these fossiliferous localities, including georeferenced positions as well as the macrofossil richness.

Palaeogeographically, the Pirabas Formation is the southeasternmost point in an area with several fossiliferous units from Central and South America in the western Central Atlantic Ocean, with geological ages around the Neogene period (Aguilera & Páes, 2012; Aguilera et al., 2013). During the greater part of this period, the Isthmus of Panama was not uplifted (O'Dea et al., 2016) and the connection between the Pacific and Atlantic Oceans allowed for an important input of nutrients from the west to the east (Schneider & Schmittner, 2006). In addition, the faunal composition of the Pirabas Formation is very similar to the Gatunian subprovince, which includes geological units of the region known as the "proto-Caribbean" (Aguilera & Páes, 2012). This dynamic promotes debate about the palaeobiogeographic origins of the different groups occurring in these subprovinces.

The vertebrate palaeofauna in the Pirabas Formation is very diverse, being represented by groups like sharks and rays (Santos & Travassos, 1960; Aguilera et al., 2017), bony fishes (Santos & Salgado, 1971; Aguilera et al., 2014), chelonians (Costa et al., 2004), crocodilians (Toledo et al., 1997; Moraes-Santos et al., 2011) and sirenians (Toledo & Domning, 1989). Among these, sirenians are the only aquatic mammals known from the Pirabas Formation, and their remains are abundant in this unit, found mainly as ribs and vertebrae. Despite this abundance, their occurrence is restricted to certain fossiliferous sites.

In relation to the records of sirenians, Távora et al. (2010) refer to the occurrence of *Dioplotherium* cf. *D. allisoni* (Kilmer, 1965) in Aricuru, in the Maracanã municipality, but the only reference cited for this locality is a study that reported only bryozoans (Távora & Fernandes, 1994). At the moment, the occurrence of sirenians in the Maracanã municipality has not been formally published. Therefore, this note addresses a new occurrence of sirenian fossils from the Pirabas Formation in the Pará state.

MATERIAL AND METHODS

The sample under study was collected during a fieldwork (in 23/03/2012) at the Praia de Fortalezinha (0° 37' 33" S, 47° 32' 30" W), Ilha de Fortalezinha, located in the northwestern part of the Maracanã municipality, Pará state, Brazil (Figure 1). The material is in a *Packstone/grainstone* rock block (Aguilera et al., 2017) found on the beach that contains several exposed segments of sirenian ribs (Figures 2A and 2B). In the laboratory, early preparation steps on the block allowed to better observe the ribs, as well as parts of a skull (Figures 2B and 2C), which will be further cleaned for a more specific identification. The assignment of the ribs and skull to the Sirenia order comes first from comparisons with previous collected material deposited into the MPEG palaeontological collection (e.g., Costa et al., 2004), abundant in some Pirabas Formation sites. Nonetheless, additional references were also used to determine the specimen (Paula-Couto, 1967; Toledo, 1989; Toledo & Domning, 1989; Domning et al., 2010), taking into consideration in this preliminary identification morphological criteria such as the rib and skull shapes. After preparation, the material was stabilised with Paraloid B72 acrylic consolidant to prevent degradation. The piece has been catalogued under the number MPEG-2427-V in the Palaeontological Collection of the Museu Paraense Emílio Goeldi (MPEG).

RESULTS AND DISCUSSION

Paula-Couto (1967) made the first formal record of sirenians in the Pirabas Formation, from material collected in the

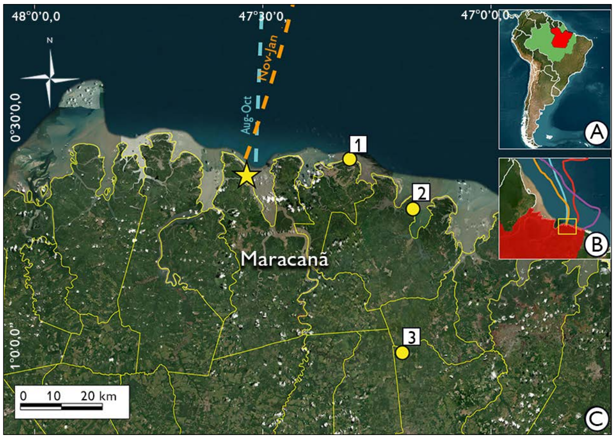

Figure 1. A) South America and in Brazil, the 'Amazônia Legal' area is given in green, with the Pará state remarked in red. The coastal zone of the Eastern Amazon is shown in (B), and the lines denote the Amazon-Tocantins delta fan oscillating intensity on the Atlantic Ocean (orange: November to January; blue: August to October; red: May to July; purple: February to April; based on Salisbury et al., 2011; Moura et al., 2016). The yellow square encloses the area shown in (C), part of the Salgado Paraense region. The Praia de Fortalezinha site (star) and other sites (circles) with sirenian occurrences are indicated: 1 = Praia do Atalaia, 2 = Ilha de Fortaleza and Ponta do Castelo, 3 = B17 mine. Map by the authors (2025).

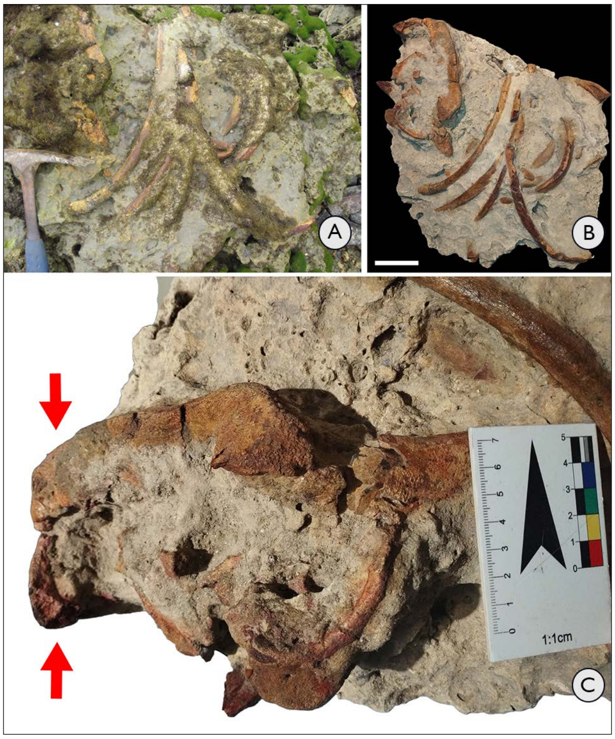

Figure 2. A) *Packstone/grainstone* block with sirenian remains, before preliminary treatment, showing only rib fragments; B) block after preliminary cleaning showing cranial remains (scale bar: 5 cm); C) detail of the cranial material, with red arrows showing the fragmented rostrum (premaxilla and incisor tooth). Images by the authors (2025).

Ilha de Fortaleza locality (type locality), at the São João de Pirabas municipality. Based on the crowns of molar teeth (one upper and one lower), he determined a new taxon named *Sirenotherium pirabense* (Paula Couto, 1967). The author also registered fragments of ribs and vertebrae from the Salinópolis municipality (cliff) and similar specimens from the Cassiano site, at the Nova Timboteua municipality, Pará.

Subsequently, Toledo (1989) collected sirenian samples from the Praia do Atalaia, at the Salinópolis municipality, including a very well preserved skull and mandible, besides vertebrae, which he attributed to *Sirenotherium pirabense*. Toledo and Domning (1989) reviewed this material and studied other cranial and post-cranial remains from this locality, and from the B5 mine of *Cimentos do Brasil* (CIBRASA), at the Capanema municipality. The authors found three taxa belonging to the same family, Dugongidae, distributed in two subfamilies: *Dioplotherium* cf. *D. allisoni* and cf. *Rytiodus*, of the Dugonginae subfamily and cf. *Metaxytherium*, Halitheriinae subfamily. *Sirenotherium pirabense* was considered by them as *nomen nudum*, since its determination does not contain diagnostic characters that justify the definition of a new taxon.

Sirenian remains are abundant in Pirabas Formation and, as in other formations, are mainly represented by vertebrae and ribs, with fragments of pachyosteoclerotic ribs being the most common fossils. However, these generally do not allow for more detailed taxonomic identification. Even teeth, which allow the determination of most fossil mammals, have limited diagnostic value in Sirenia, compared to cranial parts, particularly the cranial roof and rostrum (Domning et al., 2010). This paper records new sirenian remains to the Pirabas Formation consisting of cranial elements, a fragmented rostrum (premaxilla with upper incisor tooth, Figure 2C), which are essential for the potential taxonomic determination. An identification at least to the genus level will contribute to knowledge of the group's diversity and geographical distribution.

The number of sirenian taxa recorded in Pirabas Formation shows the high diversity of this group in a single geological formation, compared to others in Central and North America (Costa et al., 2004). Three genera were already identified for this unit, within the Dugongidae family: cf. *Rytiodus*, *Dioplotherium*, and cf. *Metaxytherium*. Among them, cf. *Rytiodus* was first known only to the Old World, and it was documented for the first time in the Americas; and *Dioplotherium*, earlier documented in North America, had its distribution extended to South America (Toledo & Domning, 1989). Neither taxon is native, and the northeastern part of Amazon would be the southern limit of *Dioplotherium* and cf. *Metaxytherium* palaeogeographic distribution, and the western limit of *Rytiodus* (Costa et al., 2004).

CONCLUSION

The new finding of sirenians in the Pirabas Formation with cranial remains of diagnostic value could potentially contribute to the specific taxonomic identification. Consequently, this will corroborate the biodiversity of the group in the northeast of the Eastern Amazon. In addition, this suggests that the group was widely distributed on the coast of the Pará state during the Neogene, justifying the presence of large predators in the region, such as sharks of the *Otodus* genus.

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AUTHORS' CONTRIBUTION

H. M. Moraes-Santos contributed to project administration, formal analysis, acquisition of funding, conceptualisation, data curation, investigation, methodology, validation, and writing (original draft, review, and editing); Z. A. S. Luz contributed to conceptualization, data curation, investigation, methodology, validation, visualisation, and writing (original draft, review, and editing); and A. P. Linhares contributed to formal analysis, conceptualisation, data curation, investigation, methodology, validation, and writing (original draft, review, and editing).

An efficient low-cost method for sampling floral visitors on down-facing flowers

Um método eficiente e de baixo custo para amostragem de visitantes florais em flores voltadas para baixo

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Abstract: Angiosperm flowers have morphological attributes that influence their interaction with pollinators, one of which is floral orientation. Pendent (down-facing) flowers are considered a protective mechanism against the loss of floral resources, such as pollen, nectar, and resin. This study proposes a new method for collecting floral visitors in *Clusia grandiflora* (Clusiaceae), a resinous species with downward-facing flowers. The Entomological Rod method consists of using a light stem (such as a buriti petiole or bamboo) pierced in the middle with a 50 ml Falcon tube attached, allowing direct capture of insects in the flowers without the need for pruning or sudden movements. Tested in Lençóis Maranhenses National Park (Maranhão, Brazil), the low-cost method demonstrated ease of application in difficult-to-access down-facing flowers.

Keywords: Pollination. Meliponini. Euglossini. Active search. Entomological rod.

Resumo: As flores de angiospermas possuem atributos morfológicos que influenciam sua interação com polinizadores, sendo um deles a orientação floral. Flores pendentes (voltadas para baixo) são consideradas um mecanismo de proteção contra a perda de recursos florais, como pólen, néctar e resina. Este estudo propõe um novo método para coleta de visitantes florais em *Clusia grandiflora* (Clusiaceae), uma espécie resinosa com flores voltadas para baixo. O método de haste entomológica consiste na utilização de uma haste leve (como pecíolo de buriti ou bambu) perfurada no meio com um tubo Falcon de 50 ml acoplado, permitindo a captura direta de insetos nas flores sem a necessidade de podas ou movimentos bruscos. Testado no Parque Nacional dos Lençóis Maranhenses (Maranhão, Brasil), o método de baixo custo demonstrou facilidade de aplicação em flores voltadas para baixo, de difícil acesso.

Palavras-chave: Polinização. Meliponini. Euglossini. Busca ativa. Haste entomológica.

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INTRODUCTION

Angiosperm flowers have various physical attributes, such as size, shape, texture, color patterns, spatial orientation, and volatile compounds. Initially, from an evolutionary point of view, these structures had the function of protecting the reproductive organs. Over time, however, they also acquired attractive functions, offering diverse floral resources for pollinators (Lima, 2000; Dafni et al., 2005), thereby becoming an important mechanism for reproductive isolation and the promotion of diversity (Grant, 1949).

Simple changes in floral morphology, such as flower orientation relative to the branch axis, can significantly influence reproductive success. Flowers oriented downward, known as down-facing flowers (Tadey & Aizen, 2001; Wang et al., 2014), represent an effective protective mechanism against the depletion of floral resources such as pollen and nectar (Ushimaru & Hyodo, 2005). Several angiosperm taxa have flowers with a pendulous orientation and offer varied features. Among them, *Clusia grandiflora* Splitg. (Clusiaceae, Figures 1A and 1B) stands out. This species is even more peculiar because it also produces resin, an uncommon feature among angiosperms (Figure 1C), which is restricted to only five genera within the order Malpighiales, *Clusia L.*, *Chrysochlamys Poepp.*, *Tovomitopsis Planch. & Triana* (Clusiaceae), *Clusiella* Planch. & Triana (Calophyllaceae), and

Figure 1. A) Branch of *Clusia grandiflora* with flower in anthesis seen from below; B) staminate flower of *C. grandiflora* seen from above; C) staminate flower of *C. grandiflora* see from below; D) the first author under a specimen of *C. grandiflora* in a shrubby restinga in the study area. Photos: Lucas C. Marinho (2023).

Dalechampia Plum. ex L. (Euphorbiaceae) (Armbruster, 1984; Langenheim, 1990; De Oliveira et al., 1999; Lokvam & Braddock, 1999; Gustafsson & Bittrich, 2003; Ribeiro et al., 2020).

A distinctive feature of many *Clusia* species is the production of floral resin. This resource, considered atypical among flowering plants, is widely used by several bee species as raw material for nest construction, waterproofing, and sanitation (Armbruster, 1984). It is assumed that resin use helps reduce the activity of pathogenic agents within nests. The bee genera most frequently observed visiting these plants include *Euglossa*, *Eulaema*, *Melipona*, and *Trigona* (Armbruster, 1984; Rech et al., 2014). Moreover, as proposed by Gustafsson and Bittrich (2003), one may speculate on the relationship between the remarkable floral morphological diversity observed in resiniferous species and resin production, regarded as key innovation driving morphological diversification in *Clusia* flowers.

Given the complexity of ecological interactions between invertebrates and plants, it is es sential to standardize collection protocols that combine representativeness, efficiency, and low cost (Araújo, 2007). For adequate faunal sampling, the simultaneous use of complementary methods capable of encompassing the diversity of habits of the species involved is recommended (Reis & Kraemer, 2013). Faunistic sampling methods can be classified as active, when the researcher actively searches for animals, or passive, when data are collected using traps or other field devices (Krug & Alves-dos-Santos, 2008). Although passive methods have already been used in studies aimed at identifying and characterizing pollinators and flower visitors (Vrdoljak & Samways, 2012), active searching remains the most common procedure, especially through sensing in flowers using entomological nets (Sakagami et al., 1967; McCravy, 2018).

Although widely recommended, active search with nets, when applied to bee surveys, requires the combination of different capture methods. This combination increases the reliability of data analysis and improves the determination of the ecological importance of the collected fauna (Krug & Alves-dos-Santos, 2008). However, specific methods for capturing bees in downward-facing flowers are still lacking, representing a gap in studies of the reproductive biology of these plants. In this context, and seeking to expand the diversity of methods for collecting invertebrates in plant reproductive biology studies, we propose a new method for capturing bees in flowers with a descending floral orientation.

MATERIAL AND METHODS

SPECIES CHARACTERIZATION

In Brazil, the genus *Clusia* is predominantly found in the Amazon and Atlantic Forest (Nascimento Jr. & Alencar, 2025). Among the morphological traits of these plants, variation in size, the arrangement of floral parts, and coloration are notable (Marinho et al., 2020). Some species have an androecium with stamens fused in different forms, as well as the presence of staminodes, which are usually responsible for providing resin as a resource to pollinators. In most cases, it is possible to observe a division between resiniferous staminodes and fertile stamens, in which either pollen or resin can be produced (Gustafsson et al., 2007; Marinho et al., 2020).

Clusia grandiflora flowers have a rotund corolla composed of five large, free, white petals with pink bases, arranged radially in an actinomorphic pattern (Figures 1B and 1C). Staminate plants have stamens connected at the base, forming a crown-like structure around the resin-secreting staminodes. Pistillate flowers are characterized by a ring of staminodes surrounding the ovary, where resin production is lower than that of staminate plants. In addition, they emit a sweet and mild fragrance, perceptible when the flowers are open (Bittrich & Amaral, 1996; Bittrich, 1997; Fernandes, 2007). Regarding size, flowers of staminate plants have an average diameter of 6.46 cm, while flowers of pistillate plants average 5.94 cm — data obtained along this project for the local plants.

OBSERVATION AREA AND CONTEXT

The entomological rod method is proposed based on a study carried out in shrubby *restinga* vegetation (*sensu* Rodrigues et al., 2019) in a natural state of conservation (Figure 1D), located in the buffer zone of the Lençóis Maranhenses National Park (PNLM, acronym in Portuguese), in the state of Maranhão, Brazil (Figure 2).

We carried out three field expeditions to the study area in May, July, and August 2023 to monitor the flowering period of *C. grandiflora*. The populations had

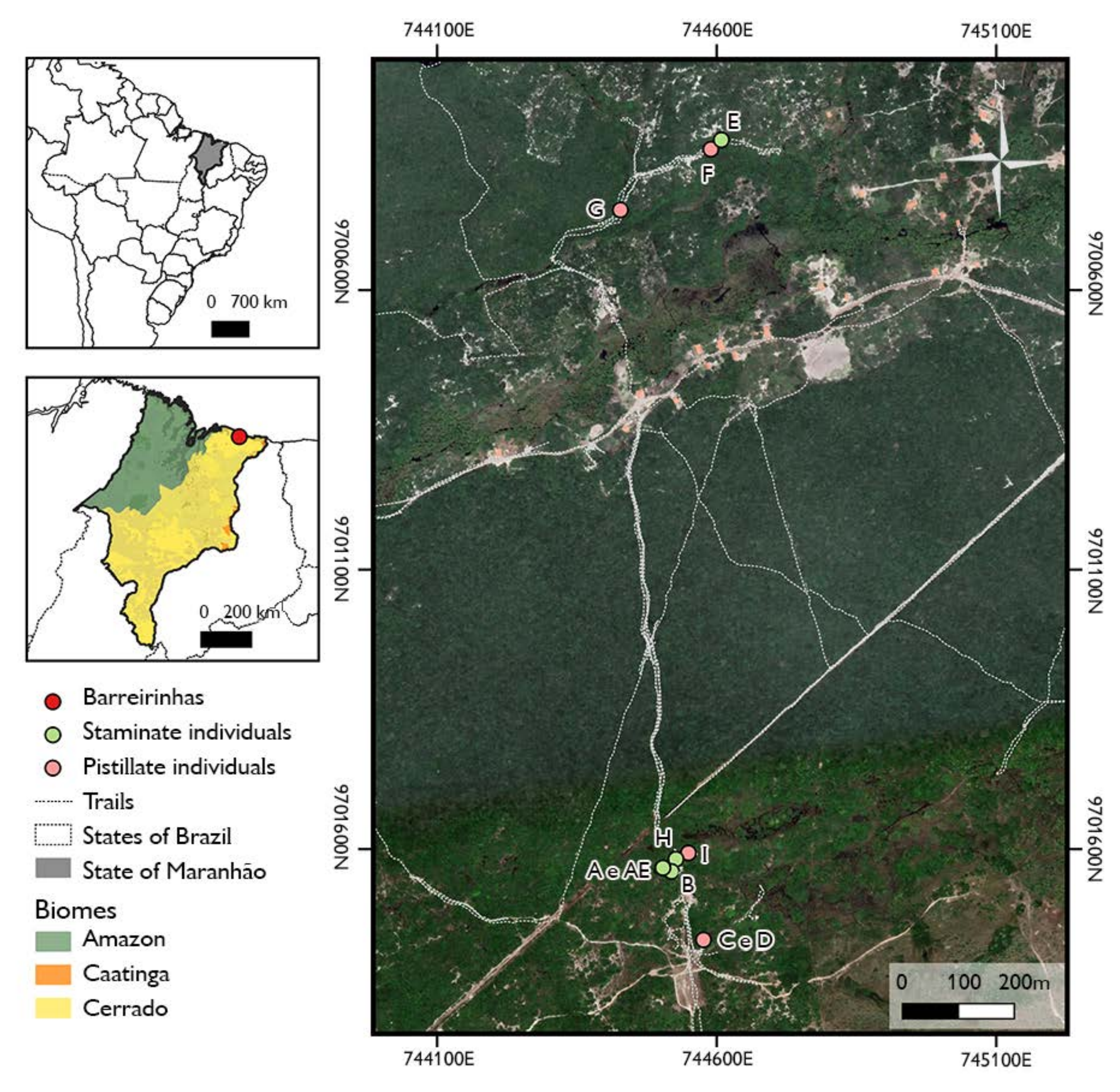

Figure 2. Map of the collection points in the natural restinga near the Atoleiro community in Barreirinhas, Maranhão, Brazil. The points indicate the locations of the monitored *Clusia grandiflora* individuals, identified by uppercase letters corresponding to the codes used on the labels. Green points represent staminate individuals, while pink points represent pistillate individuals. In the upper left corner, a map showing the location of the state of Maranhão in South America, along with a map indicating the municipality of Barreirinhas within the state (red point) and the distribution of biomes in Maranhão. Map: David B. Muniz (2023).

been previously monitored by collaborators living in the study area, who notified us of the beginning of flowering. Each field expedition consisted of two consecutive days of observations, where both techniques were used: entomological nets and rod. Each observation was conducted by four researchers (two per observed *Clusia* individual) from 5:40 AM to 5:40 PM, totaling 12 hours per day, in accordance with the region's solar cycle. To standardize data collection, each pair of observers recorded the time of each visit and the behavior of the flower visitor. Every three consecutive hours of observation, the pairs rotated the plants they were monitoring, ensuring that each pair of researchers observed all individuals for at least six hours. Pollen and resin collection behavior, the frequency of *C. grandiflora* flower visitors, and the phenological and morphological characteristics of the species were all recorded.

The flower visitors of *C. grandiflora* were initially captured with entomological nets and euthanized/stored in 50 ml Falcon tubes containing 70% alcohol. However, considering the morphology and orientation of the flowers (Figure 1B), which face the ground and are often arranged between branches (Figure 1A), this would require reverse movements (bottom-up), making handling the net, which is typically applied to upward-facing flowers, difficult. Furthermore, it would be necessary to prune branches to allow for closer contact, which would compromise the natural environment and potentially alter the number and behavior of flower visitors.

Due to the difficulty and low efficiency of using entomological nets to capture bee visitors of *C. grandiflora*, we developed a device consisting of a long, lightweight rod made from a buriti (*Mauritia flexuosa* L.f.) petiole, with a 50 ml Falcon tube attached to its upper end, previously perforated for easy attachment (Figures 3 and 4). The buriti petiole was chosen based on its local availability, as the species is abundant in the region. However, the method also allows for replacement with bamboo rods, as illustrated in Figure 3, or other lightweight materials. This setup allows safe access to the flowers and accurate collection of visitors.

RESULTS AND DISCUSSION

During the study, 84 flower visitors were collected, the vast majority (66 specimens) were captured using the entomological rod, while only 18 were collected using entomological nets. Of the 84 individuals recorded on *C. grandiflora* flowers, most were observed on staminate flowers and belonged to the order Hymenoptera. Other orders, such as Coleoptera and Hemiptera, were also recorded but in smaller numbers. At present, it is not possible to assess the efficiency of the method by comparing our results with other studies on floral visitors of *Clusia* — for example, Bittrich and Amaral (1996, 1997) and Kaminski and Absy (2006) — since those works did not report the abundance of individuals, but only species composition.

From the order Hymenoptera, 78 bees (Apidae) and one ant (Formicidae) were recorded visiting *C. grandiflora* flowers. From the other orders, five individuals were observed, two of which belonged to the subfamily Eumolpinae (Coleoptera: Chrysomelidae) and moved across all parts of the pistillate plant, including the flowers. Although the proposed method, entomological rod, has not been tested with insect orders other than Hymenoptera, Coleoptera, and Hemiptera, in principle it can be applied to any specimen that can be captured in a liquid medium, such as Diptera, Orthoptera, Araneae, among others. However, for certain groups, such as mosquitoes (Diptera: Culicidae), butterflies, and moths (Lepidoptera), the method is not suitable, since preservation in liquid medium is not recommended. These insects are quite fragile, bearing long setae and scales that are easily damaged or lost in such media. The absence of these structures often makes taxonomic identification unfeasible (Almeida et al., 2024).

Our results show that most bee species captured using the entomological rod method are small to medium in size (2-15 mm). This outcome is directly related to the

Figure 3. Graphic diagram showing the application of the entomological rod method in the capturing of floral visitors of *Clusia grandiflora*. Illustration: Victor E. Cantanheide (2025).

fact that the tribe Meliponini was the most abundant and diverse in our sampling. However, the method is not limited to bees of this size range, as a specimen of *Eulaema* (Euglossini), a genus whose species can exceed 30 mm in length (large-sized), was also collected with this method. If the researcher considers the opening diameter of a 50 ml Falcon tube (30 mm) insufficient for the target specimens of their study, the method can be easily adapted to larger diameter containers, thereby better accommodating the body size of the floral visitors in question.

The new method allowed for quick and accurate captures, directly on the stamens or resin pit of *C. grandiflora*, with minimal disturbance to the environment and avoiding sudden movements that could facilitate the insect's escape. Among the main advantages of the methods include its low cost, accessible materials, and efficiency in difficult-to-reach environments where nets are impractical. The method reduces specimen loss and optimizes collection time.

To prevent the dissolution of resin collected and transported in the corbiculae of bees, caused by the

Figure 4. Entomological rod developed for capturing floral visitors to *Clusia grandiflora* flowers. Photos: Lucas C. Marinho (2023).

alcohol present in the Falcon tube, a factor that may compromise chemical analyses or the quantification of resin transported by pollinators, especially in resiniferous species, we propose replacing alcohol with water containing a neutral detergent. This solution reduces the surface tension of the water and allows the insects to be retained without dissolving the resin.

CONCLUSION

The method proposed here proved to be effective for collecting floral visitors from down-facing flowers, especially in conditions where netting is difficult to access. Its application can complement traditional sampling methods, contributing to studies of pollination ecology and plant reproductive biology in natural environments.

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AUTHORS' CONTRIBUTION

H. C. Andrade contributed to project administration and writing (original draft, review, and editing), formal analysis, investigation, and data curation; M. E. S. David-Silva contributed to data collection and writing (review); D. B. Muniz contributed to data collection, formal analysis, investigation, project administration and writing (original draft, review, and editing); and L. C. Marinho contributed to supervision, funding acquisition, project administration and writing (original draft, review, and editing).

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1. Lagarto com 4 patas minúsculas 2
   Lagarto com 4 patas bem desenvolvidas 3
2. Dígitos geralmente sem unhas, dorsais lisas .... *Bachia flavescens*
   Dígitos com unhas, dorsais quilhadas .... *Bachia panoplia*
3. Mãos com apenas 4 dedos 4
   Mãos com 5 dedos 5
4. Escamas dorsais lisas .... *Gymnophthalmus underwoodii*
   Escamas dorsais quilhadas .... *Amapasaurus tetradactylus*
5. Cabeça com grandes placas 6
   Cabeça com escamas pequenas 7
6. Placas posteriores da cabeça formam uma linha redonda .... *Alopoglossus angulatus*
   Placas posteriores da cabeça formam uma linha reta .... *Arthrosaura kockii*
7. Etc.
   Etc.

Pede-se destacar termos ou expressões por meio de aspas simples. Apenas termos científicos latinizados ou em língua estrangeira devem constar em itálico. Observar cuidadosamente as regras de nomenclatura científica, assim como abreviaturas e convenções adotadas em disciplinas especializadas. Os artigos deverão seguir as recomendações da APA 7th Edition - Citation Guide (https://apastyle.apa.org/style-grammar-guidelines/references/examples) para uso e apresentação de citações e de referências. Todas as obras citadas ao longo do texto devem estar corretamente referenciadas ao final do artigo.

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Título – No idioma do texto e em inglês (quando este não for o idioma do texto). Deve ser escrito em caixa baixa, em negrito, centralizado na página.

Resumo e Abstract – Texto em um único parágrafo, ressaltando os objetivos, métodos e conclusões do trabalho, com, no máximo, duzentas palavras, no idioma do texto (Resumo) e em inglês (Abstract). A versão para o inglês é de responsabilidade do(s) autor(es).

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Material e métodos – Exposição clara dos métodos e procedimentos de pesquisa e de análise de dados. Técnicas já publicadas devem ser apenas citadas e não descritas. Termos científicos, incluindo espécies animais e vegetais, devem ser indicados de maneira correta e completa (nome, autor e ano de descrição).

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