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A multimethodological approach for vertebrate endemism and potential priority regions for conservation in the state of Espírito Santo, southeastern Brazil

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SUMMARY

The selection of strategic conservation areas is a complex undertaking that requires the integration of various elements, including planning, indicators, and mapping. An extensive evaluation of vertebrate distribution patterns in Espírito Santo state, southern Brazil, revealed the existence of four consensus areas defined by two endemism methods that can be designated as priority regions for multitaxa conservation. A brief remark is made regarding the importance of endemic areas and biogeographic units in the definition of priority areas for conservation.

INTRODUCTION

Biogeography studies the relationship between the distribution patterns of organisms and the events that shaped the biota. A fundamental concept in biogeography is the existence of areas of endemism, basic biogeographic units characterized by the presence of species of restricted distribution that share a congruent biogeographical, evolutionary and ecological background, thus corresponding to biogeographic homologies (Platnick 1991, Morrone 1994, 2014, Escalante 2015). Areas of endemism play a pivotal role in conservation biology (Fattorini 2017). The delineation of these areas is of paramount importance for the formulation of public conservation policies, for the allocation of resources and the optimization of efforts (Martínez-Hernández et al. 2015, Avila-Torres et al. 2023).

The state of Espírito Santo encompasses more than 120 conservation units under federal, state, municipal, and private administration. These units are classified as integral protection and sustainable use (INMA 2021). The largest of these is the federally administered Reserva Biológica de Sooretama (278 km²) (Rosa et al. 2019), which, together with the private Reserva Natural Vale and other contiguous protected areas, covers an area of approximately 500 km². Also significant is the Parque Nacional do Caparaó, located on the border between the states of Espírito Santo and Minas Gerais, with an area of approximately 257 km². The protected areas in Espírito Santo collectively encompass approximately 10.5% of the remaining Atlantic Forest fragments and only 4.5% of the state (Rosa et al. 2019, INMA 2021). This represents a relatively modest level of protection in comparison to the significant number of 1,874 threatened taxa, the majority of which are not under the protection of a conservation unit (Fraga et al. 2019).

The objective of this study is to identify priority areas for the conservation of vertebrate species in the state of Espírito Santo, southeastern Brazil, based on multiple analyses

of endemism, the presence of threatened taxa and the current availability of protected areas. This study was conducted as part of research activities within the Conservation Biogeography course, with the post-graduate team.

MATERIALS AND METHODS

Study area

The state of Espírito Santo is situated in the southeastern region of Brazil, with a territorial extension of approximately 46,074 km², (IBGE 2023). The natural vegetation in the study area is composed of the Atlantic Forest and adjacent pioneer formations such as the coastal sand formations, mangroves and coastal flooded grasslands. There are some differences in the Atlantic Forest formations along the area. In the northern region, the dense ombrophilous forest is the predominant vegetation, while the southern region is dominated by a mixed ombrophilous forest (Rizzini 1979, Coimbra Filho and Câmara 1996, de Paula 2006).

The northern domain corresponds to the coastal tablelands of the Barreiras group, which exhibit mild and undulating low-altitude relief (Braun and Ramalho 1980, Garay and Rizzini 2004, Sarmento-Soares and Martins-Pinheiro 2012, 2014). The central-southern region of the state is characterized by the presence of highlands and mountains, which are extensions of the Serra da Mantiqueira mountain range. These include the Serra do Caparaó and Serra do Castelo. The topography of the area encompasses the highest elevations within the Atlantic Forest biome, including the Pico da Bandeira at 2,891 m, situated on the slopes of the Caparaó Mountains (Novo et al. 2011). The hydrographic network is comprised of 13 basins and microbasins of different sizes. Of these, the drainages of the Rio Doce, Rio Itaúnas, Rio São Mateus, and Rio Itapemirim are of particular significance (Sarmento-Soares and Martins-Pinheiro 2012, 2013). The contrasting relief along the study area is reflected in its biodiversity, with different sets of endemic

species in distinct regions of the state (Vieira-Guimarães et al. 2023).

Data compilation

The occurrence and distribution of vertebrate species in the state of Espírito Santo were evaluated based on published lists for freshwater fish (Sarmiento-Soares and Martins-Pinheiro 2010, 2012, 2013, 2014, Sarmiento-Soares et al. 2012, Vieira-Guimarães et al. 2023), amphibians (Almeida et al. 2011, Ferreira et al. 2019, Silva-Soares and Norbertino 2023), reptiles (Costa and Bérnils 2018, Costa et al. 2021, Silva-Soares and Santiago 2023), birds (Simon 2009) and mammals (Moreira et al. 2008, Mendes et al. 2010, Vela-Ulian et al. 2021). We compiled 60,455 georeferenced records for 1,225 species of vertebrates deposited in museums, direct observations, and audiovisual recordings from the Species Link (<https://smlink.cria.org.br>), GBIF (<https://www.gbif.org>), SiBBR (<https://sibbr.gov.br>) and data repositories. These include 3,108 records of 123 freshwater fish species (Fig. 1A-B), 1,763 records of 143 amphibians (Fig. 2A-B), 1,230 records of 114 reptiles (Fig. 3A-B), 52,401 records of 652 birds (Fig. 4A-B), 1,075 records of 116 non-flying mammals (excluding Cetacea) (Fig. 5A-B), and 878 records of 77 bats (Fig. 6A-B). Complementary information on the occurrence of species was obtained from specific databases such as the Cornell Lab of Ornithology – eBird (<https://ebird.org>), WikiAves (<https://wikiaves.com.br>), AmphibiaWeb (<https://amphibiaweb.org>), Herpeto Capixaba (<https://www.herpetocapixaba.com.br>), The Reptile Database (<http://www.reptile-database.org>), Fishbase (Froese and Pauly 2024) and Catalog of Fishes (Fricke et al. 2024). The threat levels of the species follow Fraga et al. (2019). All datasets and additional information on the areas of endemism defined here are available at https://www.researchgate.net/publication/380245899_Vertebrate_database_ES_southeastern_Brazil.

Areas of endemism and priority areas

The identification of endemism areas in Espírito Santo was carried out using the NDM/VNDM v. 3.1 software (Szumik et al. 2002, Szumik & Goloboff 2004). This analysis highlights areas with congruent species distributions (Szumik et al. 2002, Goloboff 2012, Aagesen et al. 2013, Szumik and Goloboff 2015), using geographic coordinates that are transformed into presence or absence in the grid cells. The NDM software calculates endemism indices for each species, which are summed to give an endemism score for each area/cell on the map presented in the VNDM software. The endemism index results from the evaluation of the total distribution of the taxon and the fit of the distribution to the area. The areas of endemism are further consolidated into consensus areas, where the resulting grids delineate endemism hotspots. The following parameters were used: cell size = 0.20°x0.20°, minimum number of endemic species = 2; minimum score for species = 0.5; minimum value of saved sets = 2.0; minimum percentage of unique species retained = 50%; flexible consensus of 25% species similarity; search repeated 100 times. The remaining settings follow the program defaults.

We also conducted a Geographic Interpolation of Endemism (GIE) in the BioDinamica library (Oliveira et al. 2019) of the Dinamica-EGO software (Soares-Filho et al. 2002). This analysis delineates concentrations of restricted range species through kernel interpolations of the distributions of several taxa (Oliveira et al. 2015). The following parameters were used: six size classes = 0-25km, 25-50km, 50-75km, 75-100km, 100-150km, 150-200km, raster grid size = 0.03, using a delimitation mask of the state of Espírito Santo. The remaining settings follow the program defaults. For both analyses we considered endemic areas delineated not only by species exclusive to the state but also by any taxa exhibiting restricted distribution within the study area, regardless of their occurrence in other regions.

We overlaid the results of the maps generated by NDM/VNDM (simple overlay) and GIE (overlay through algorithm in the Biodinamica software) and compared them with the availability of conservation units, priority areas according to MMA (2018), and the presence of threatened species in all taxonomic groups. This was done to generate a consensus map that aims to (a) identify potential areas that lack conservation measures and (b) reinforce the necessity of continuous conservation efforts in areas that already present conservation units. The output maps were produced using the QGIS software (Qgis Development Team 2022).

RESULTS AND DISCUSSION

Freshwater fishes

The results of the GIE analysis (Fig. 1) revealed clusters of endemic species distributed throughout the state, with the greatest concentration observed in the central-southern portion, spanning from the highlands in the central region to the Caparaó mountains (Fig. 1C). The NDM/VNDM analysis delineated seven areas of endemism, which were consolidated into four consensus areas (Fig. 1D). Two of these consensus areas exhibit partial overlap, forming a possible diffuse transition zone where there is a gradual replacement of the biotic components of each area (Ferro and Morrone 2014, Noguera-Urbano and Escalante 2015). This biogeographic outcome is similar for other vertebrate groups presented here. A previous analysis of endemism conducted by Vieira-Guimarães et al. (2023) for freshwater fishes in the study area is expanded upon in this paper, as two additional areas of endemism were identified through the NDM/VNDM method. The overlay of the results from both methods of endemism indicates congruence in the delimited areas, with one or other area differing between these findings (Fig. 1E).

Out of the 123 freshwater fish species listed here for the state of Espírito Santo, 18 are threatened (14.6%) while only seven (5.7%)

have populations safeguarded within protected areas (Hostim-Silva et al. 2019, Fraga et al. 2019). Also, a considerable number of populations of these threatened species are situated outside both the areas of endemism and the priority areas defined by the MMA (2018). The situation for fish and other freshwater organisms is even more dramatic, as conservation units frequently prioritize terrestrial fauna, thereby neglecting the protection of crucial areas for aquatic fauna, such as headwater streams (Sarmiento-Soares and Martins-Pinheiro 2017, Azevedo-Santos et al. 2019). These streams are home to numerous endemic and sensitive species that are vulnerable to anthropogenic impacts, including species introductions, habitat alterations, aquatic pollution, hydropower dam construction, and the removal of riparian vegetation (Castro 1999, Dudgeon et al. 2006, Menezes et al. 2007, Azevedo-Santos et al. 2019).

Amphibians

The GIE output (Fig. 2) shows an area of higher concentration of species with restricted distribution in the central mountainous region and three areas of less intensity in the north and south of the state (Fig. 2C). The NDM/VNDM analysis pointed to seven areas of endemism united in four consensus areas (Fig. 2D), two of which are partially overlapping, presenting distinct sets of species. The results of the two analyses, when superimposed, indicate partial congruence (Fig. 2E).

The state of Espírito Santo is home to 166 species of amphibians (Silva-Soares and Norbertino 2023). Our list includes 143 species, of which 17 (11.9%) are threatened (Ferreira et al. 2019, Fraga et al. 2019). Most of the records of endangered species are concentrated in the mountainous region of the center of the state, which is also the most sampled area in Espírito Santo for several groups of organisms. The municipality of Santa Teresa, one of the main research centers for the biodiversity of the

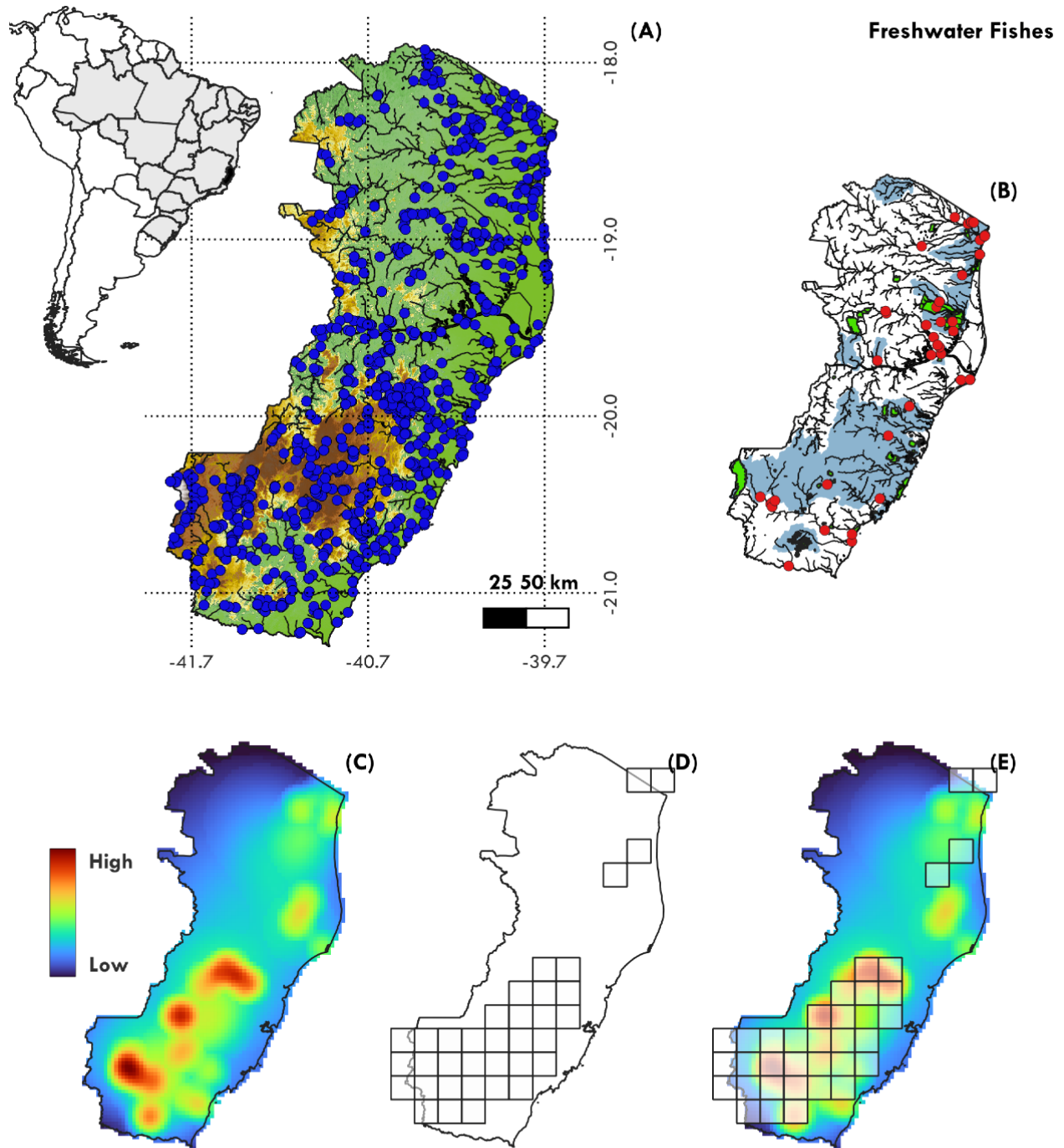


Figure 1. Areas of endemism for freshwater fishes in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

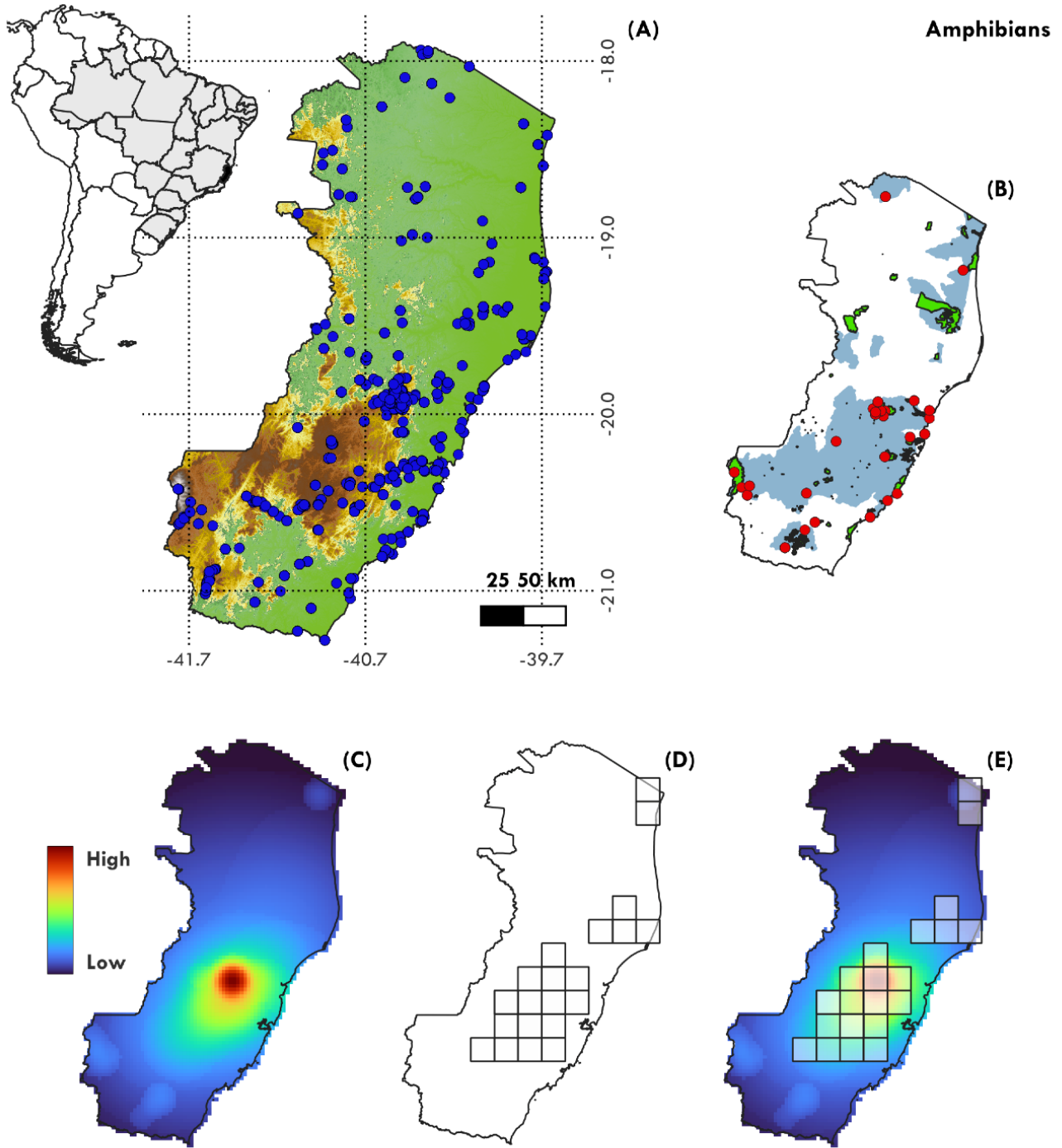


Figure 2. Areas of endemism for amphibians in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

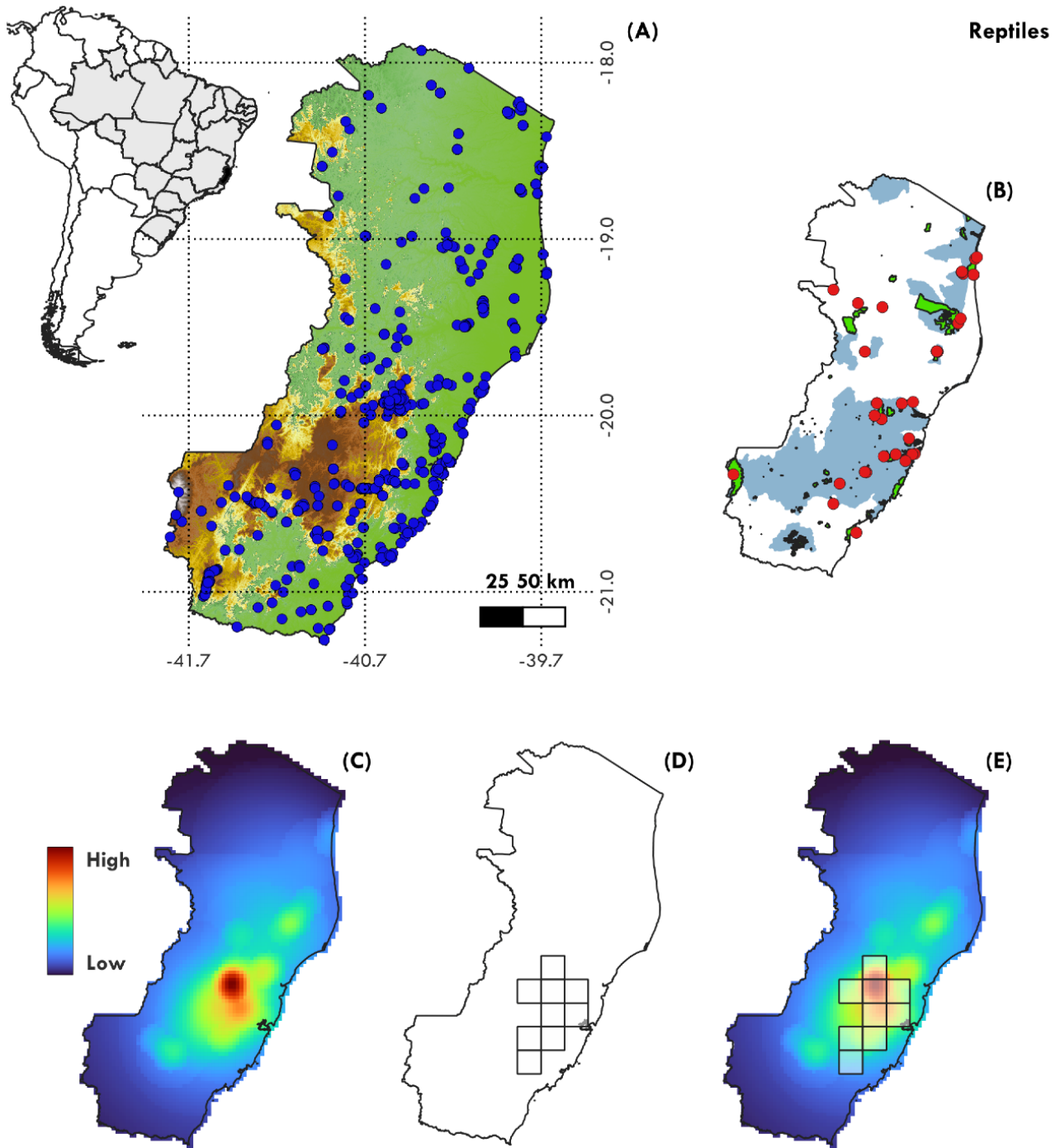


Figure 3. Areas of endemism for reptiles in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

Atlantic Forest, is characterized by high taxonomic, functional, and phylogenetic diversity of amphibians (Almeida et al. 2011, Campos et al. 2017, Ferreira et al. 2019, Lourenço-de-Moraes et al. 2019), a factor that significantly influenced the presence of areas of endemism identified in this area. Recent syntheses, as a result of data collected over more than a decade, include a list of 108 amphibian species for this municipality alone (Ferreira et al. 2019). In a slightly optimistic scenario, the conservation units of Espírito Santo are home to populations of 75% of the threatened amphibian species in the state (Ferreira et al. 2019), and almost all the records of endangered amphibians considered here are found in the priority areas already delimited by the MMA (2018) or in the areas of endemism pointed out in this study. This suggests that efforts for the implementation or continued maintenance of protected areas in these regions have already been indicated and are reinforced here. These efforts are necessary, since the most deleterious factors for amphibian fauna, such as habitat loss and fragmentation due to agricultural purposes (Luedtke et al. 2023), occur on a large scale in Espírito Santo territory.

Reptiles

The GIE analysis (Fig. 3) identified a large area with a higher concentration of restricted species in central Espírito Santo, with clusters of lower intensity to the north in the Linhares-Sooretama complex, and to the south of this focal area (Fig. 3C). Similarly, the NDM/VNDM analysis delineated seven areas of endemism that converged into one consensus area, also situated in the mountainous central region of the state (Fig. 3D). Both methods yield congruent results (Fig. 3E), suggesting that this region can be considered a cohesive biogeographic unit for reptiles in Espírito Santo, based on traditionally defined parameters (Platnick 1991, Morrone 1994).

Espírito Santo is home to 134 reptile species (Silva-Soares and Santiago 2023). In this study, we consider records of 114 species, of

which 23 are considered threatened, particularly snakes and sea turtles (Costa and Bérnils 2018, Bérnils et al. 2019, Fraga et al. 2019). Most of the records of these threatened species are concentrated in the central and metropolitan regions of the state, notably within areas prioritized by the MMA (2018). However, very few populations of these species are adequately protected. According to Bérnils et al. (2019), only 19% of the endangered reptile records in Espírito Santo are within conservation units, a concerning statistic given the various threats facing these animals in the state, including wildlife trafficking, hunting, deforestation of native forest remnants, and the limited availability of high-quality water sources.

Birds

The GIE analysis (Fig. 4) reveals two significant concentrations of species with restricted distribution in Espírito Santo: one situated in the central mountainous region extending southwestward, a pattern observed in other vertebrate taxa, and another in the north of the Rio Doce basin, within the Linhares-Sooretama complex (Fig. 4C). Two additional, albeit less intense, concentrations occur in the Caparaó mountains region, in southern Espírito Santo, and in the Itaúnas area in the north. This finding aligns with the results of the NDM/VNDM method, which identified 28 highly overlapping areas of endemism consolidated into eight consensus areas (Fig. 4D) in the same regions highlighted by the GIE, indicating congruence between these findings (Fig. 4E).

The study of bird diversity and conservation in Espírito Santo began relatively recently, with the first comprehensive survey conducted by naturalist Augusto Ruschi in 1953 (Ruschi 1953), documenting the occurrence of 636 species. Subsequent assessments categorized 81 species as endangered in the state (IEMA 2005), and a later compilation estimated the presence of 654 species (Simon 2009). In our study, we considered 652 species, with 124 (19%) classified as threatened according to

recent assessments (Chaves et al. 2019, Fraga et al. 2019). The majority of these endangered species are located within priority areas designated by the MMA (2018). However, it is worth noting that only 30% of the recorded endangered bird species in Espírito Santo are currently protected within conservation units, as highlighted by Chaves et al. (2019). Birds play a crucial ecological role, although their value is challenging to quantify in economic terms (Whelan et al. 2010). Nevertheless, recent studies indicate a concerning trend of declining bird populations, attributed to habitat fragmentation and climate change, with significant implications for ecosystem functioning (Souza et al. 2011, Chaves et al. 2019, Rosenberg et al. 2019).

Non-flying mammals

The GIE result (Fig. 5) reveals two significant and adjacent areas hosting endemic species within the central mountainous region of the state, alongside a third area of lesser extent and intensity within the Linhares-Sooretama complex (Fig. 5C). On the other hand, the NDM/VNDM analysis delineated five endemic areas for non-flying mammals, consolidating into three consensus regions. These findings corroborate the central region identified by the GIE and identify an additional area within the Caparaó mountains (Figs 5D, 5E).

The Atlantic Forest vegetation, which previously covered 90% of the territory of the state of Espírito Santo, has been reduced to small fragments in conservation units or private properties. Despite this decline, the state remains a haven for diverse mammal species and other groups, hosting roughly 50% of the vertebrate biodiversity of the biome (Moreira et al. 2008, Pinto et al. 2009, Ferregueti et al. 2014). Relevant areas in Espírito Santo include the mountains of the Caparaó massif and the central highlands of the state and the Linhares-Sooretama complex. The latter is especially vital for fostering connectivity and facilitating the recovery of forest fragments between adjacent

conservation parcels (Carbone and Gittleman 2002, Gatti et al. 2006, Saura et al. 2014, Gatti et al. 2017, de la Torre et al. 2017, Costa et al. 2019). However, out of the 116 non-volant mammal species considered in this study, 28 (24.1%) are threatened (Fraga et al. 2019), with approximately 48% of the records of these vulnerable mammals (including bats) outside protected areas (Costa et al. 2019). This renders these species more susceptible to the primary risk faced by the world's richest mammalian fauna: habitat loss, fragmentation, and alterations in natural landscapes, prevalent in the heavily human-altered environments of Espírito Santo (Mittermeier et al. 1997, Costa et al. 2005, Paglia et al. 2012, ICMBio 2018, Quintela et al. 2020).

Bats

The outcome of the GIE analysis (Fig. 6) reveals a significant concentration of species with restricted distribution in two distinct areas of the state: one spanning from the Linhares-Sooretama complex to the Rio Doce, and the other situated in the uplands of the central region (Fig. 6C). This aligns with numerous studies highlighting these two regions, particularly the Linhares-Sooretama area, as highly significant for bat diversity in the state (Mendes et al. 2010, Peracchi et al. 2011, Pimenta 2013, Hoppe et al. 2014, Gnocchi et al. 2019, Vela-Ulián et al. 2021). The results of the NDM/VNDM analysis for this group corroborate those of the GIE, as evidenced by the overlap of their outputs (Figs 6D, 6E).

The order Chiroptera ranks as the second largest group of mammals, presenting approximately 1400 species (Kruskop, 2021). In Brazil, bats exhibit remarkable diversity, with 182 species documented (Garbino et al. 2022), 122 of which inhabit the Atlantic Forest biome (Figueiredo et al. 2021). Within Espírito Santo, 88 bat species have been recorded (Vela-Ulián et al. 2023), representing 48.6% of Brazil's total and 72.1% of those found in the Atlantic Forest

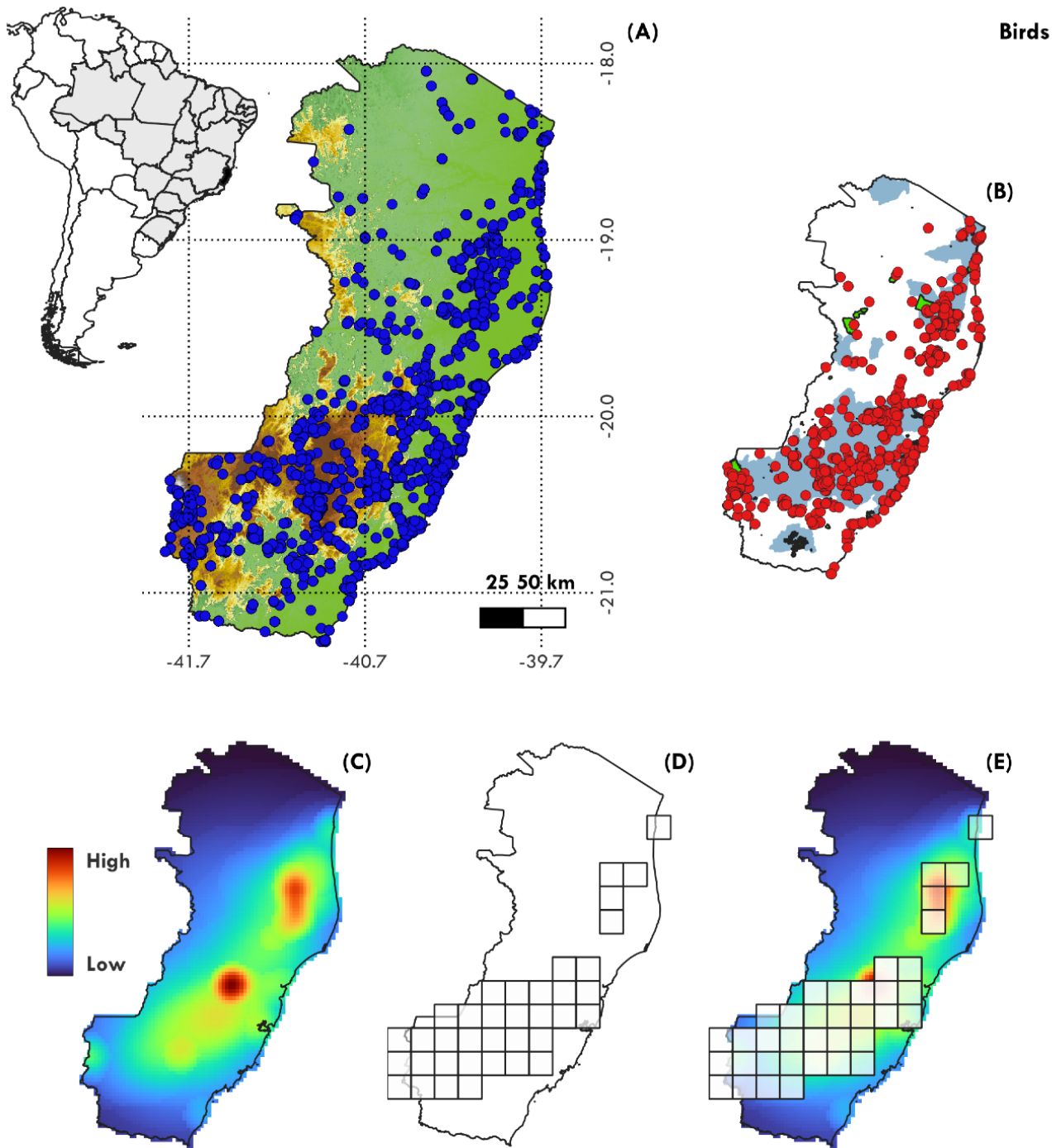


Figure 4. Areas of endemism for birds in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

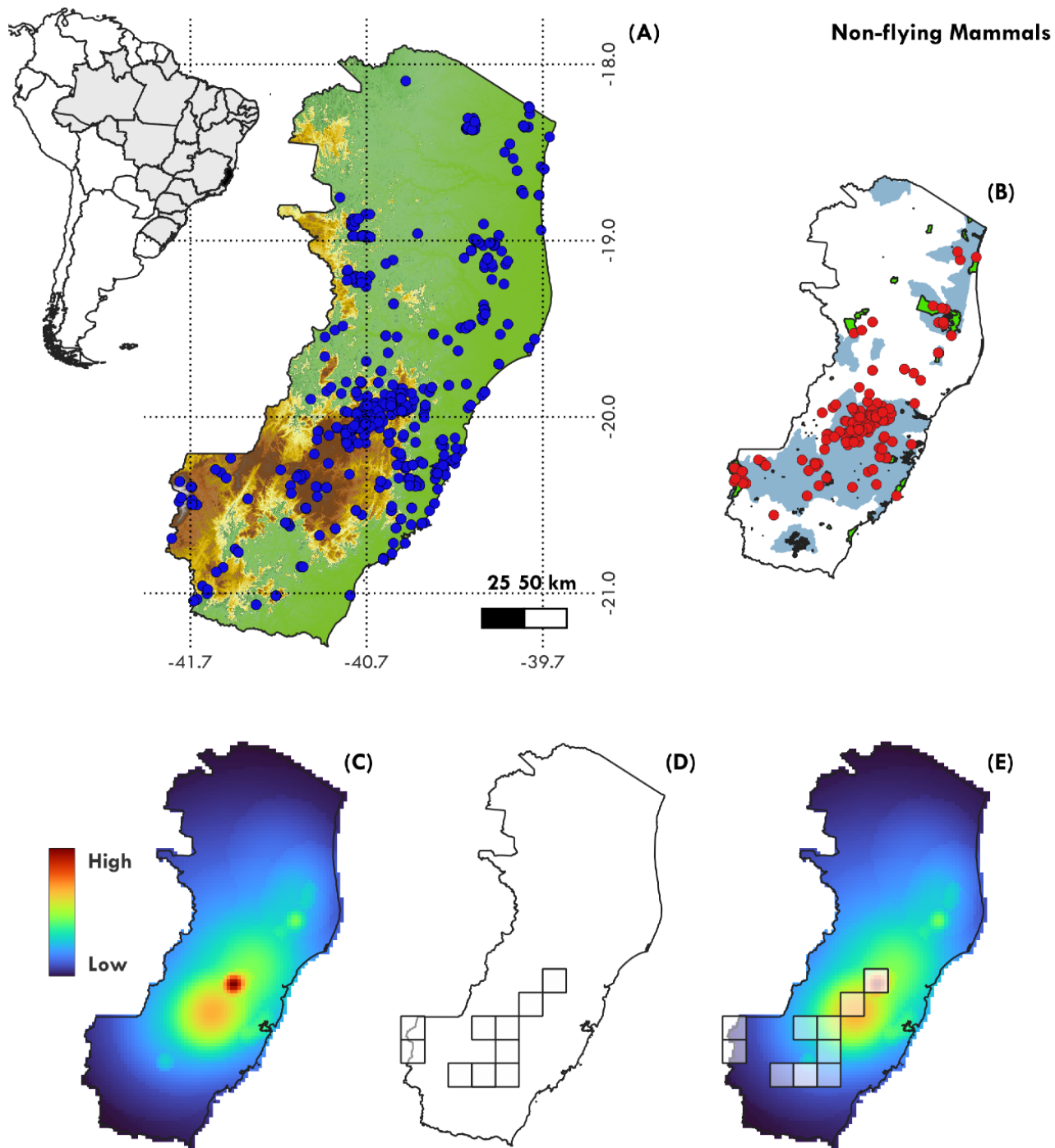


Figure 5. Areas of endemism for non-flying mammals in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

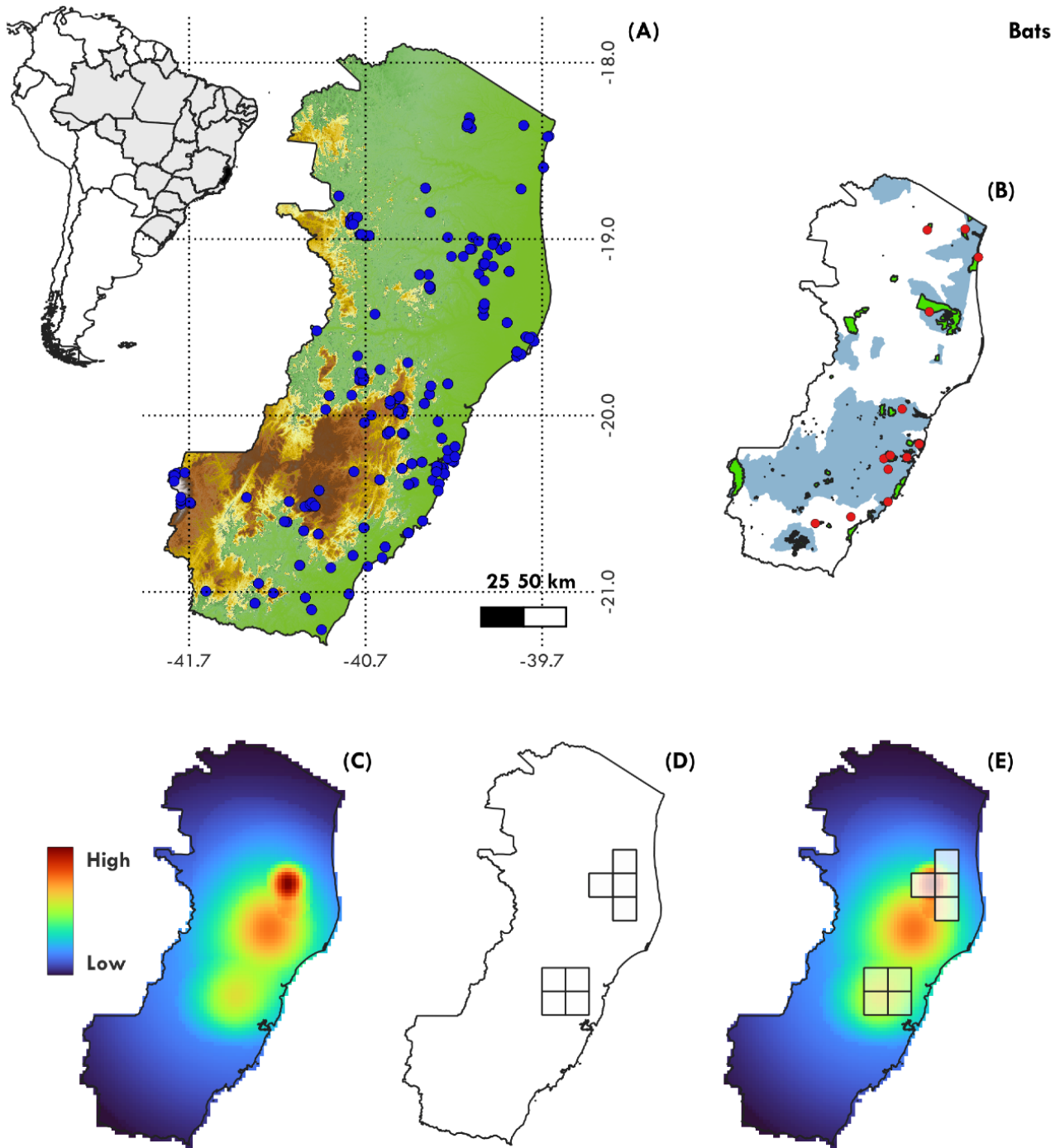


Figure 6. Areas of endemism for bats in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) GIE output; (D) NDM output; (E) Overlay of the GIE + NDM outputs.

(Figueiredo et al. 2021, Garbino et al. 2022). The state also harbors four of the nine endemic bat

species in this biome (Vela-Ulian et al. 2021, 2023). Our compilation lists 77 bat species for

the state, with five (6.5%) classified as threatened (Costa et al. 2019, Fraga et al. 2019). Primary threats to bat populations include climate change, deforestation, roadkill incidents, and forest conversion to agriculture, all posing significant risks to regions with high endemism (Frick et al. 2020, Damásio et al. 2021). Therefore, it's crucial to recognize that bats, due to their adaptability in utilizing food resources—such as fruits, insects, nectar, and small vertebrates (Kalko 1998, Altringham 2011)—play vital ecological roles within their habitats, including pollination, seed dispersal, pest control, and nutrient transportation (Altringham 2011, Kunz 2011, Vale et al. 2023). Understanding these functions underscores the importance of promptly addressing key threats to bat biodiversity to develop effective conservation strategies (Marchese 2015).

Consensus and priority areas for conservation

The overlap of results from all analyses, coupled with the presence of endangered species records and the existence of protected areas, delineates a virtually uninterrupted stretch of regions spanning from the north to the south of the state (Fig. 7). This outcome closely mirrors the priority conservation areas identified by the MMA (2018). Consequently, we propose focusing or strengthening conservation efforts in four regions of the state: Northeastern Coast, Linhares-Sooretama-Lower Rio Doce Complex, Central-Southern Highlands and Vitória surroundings, and Caparaó Highlands.

I. Northeastern Coast: A coastal area in the north of the state, which is home to small forest fragments and conservation units, such as the Parque Estadual de Itaúnas and the Área de Proteção Ambiental de Conceição da Barra, as well as the Reserva Biológica de Córrego Grande. These areas, situated predominantly in the municipality of Conceição da Barra, harbor a significant concentration of species with restricted ranges or endangered statuses. Often, these species share habitats with areas in the

southern region of Bahia. Within this priority area lie various forest fragments where endangered species of freshwater fish, such as *Acentronichthys leptos* Eigenmann & Eigenmann 1889, *Mimagoniates sylvicola* Menezes & Weitzman 1990, *Rachoviscus graciliceps* Weitzman & Cruz 1981, *Xenurolebias myersi* (Carvalho 1971); the amphibian *Proceratophrys moehringi* Weygoldt & Peixoto 1985; the reptiles *Ameivula nativo* (Rocha, Bergallo & Peccinini-Seale 1997) and *Caiman latirostris* (Daudin 1801); the birds *Amazona rhodocorytha* (Salvadori 1890), *Circus buffoni* (Gmelin 1788), *Crypturellus noctivagus* (Wied-Neuwied 1820), *Mimus gilvus* (Vieillot 1808), among others; and the mammals *Leopardus pardalis* (Linnaeus 1758) and *Sapajus robustus* (Kuhl 1820) (Simon 2009, Costa and Bérnils 2018, Bérnils et al. 2019, Chaves et al. 2019, Costa et al. 2019, Ferreira et al. 2019, Fraga et al. 2019, Hostim-Silva et al. 2019, Sarmiento-Soares and Martins-Pinheiro 2013, 2017). The main threats to organisms in this endemicity area include aggressive agricultural land use, eucalyptus plantation, deforestation, and low water availability (Sarmiento-Soares and Martins-Pinheiro 2017, Rosa et al. 2019).

II. Linhares-Sooretama-Lower Rio Doce Complex: This region is home to a significant diversity of species, including endemic and endangered, for various groups of organisms, especially birds and mammals. The priority area extends from the largest forest remnant in lowlands of Espírito Santo, the Reserva Biológica de Sooretama to the neighborhood of the Floresta Nacional de Goytacazes, covering several forest fragments in the municipalities of Jaguaré, Sooretama and Linhares. Threatened species for this region include the freshwater fish *Brycon dulcis* Lima & Vieira 2017, *Rachoviscus graciliceps*, *Scleromystax virgulatus* (Nijssen & Isbrücker 1980), *Xenurolebias izecksohni* (Da Cruz 1983), among others; the reptiles *Ameivula nativo*, *Apostolepis longicaudata* Gomes 1921,

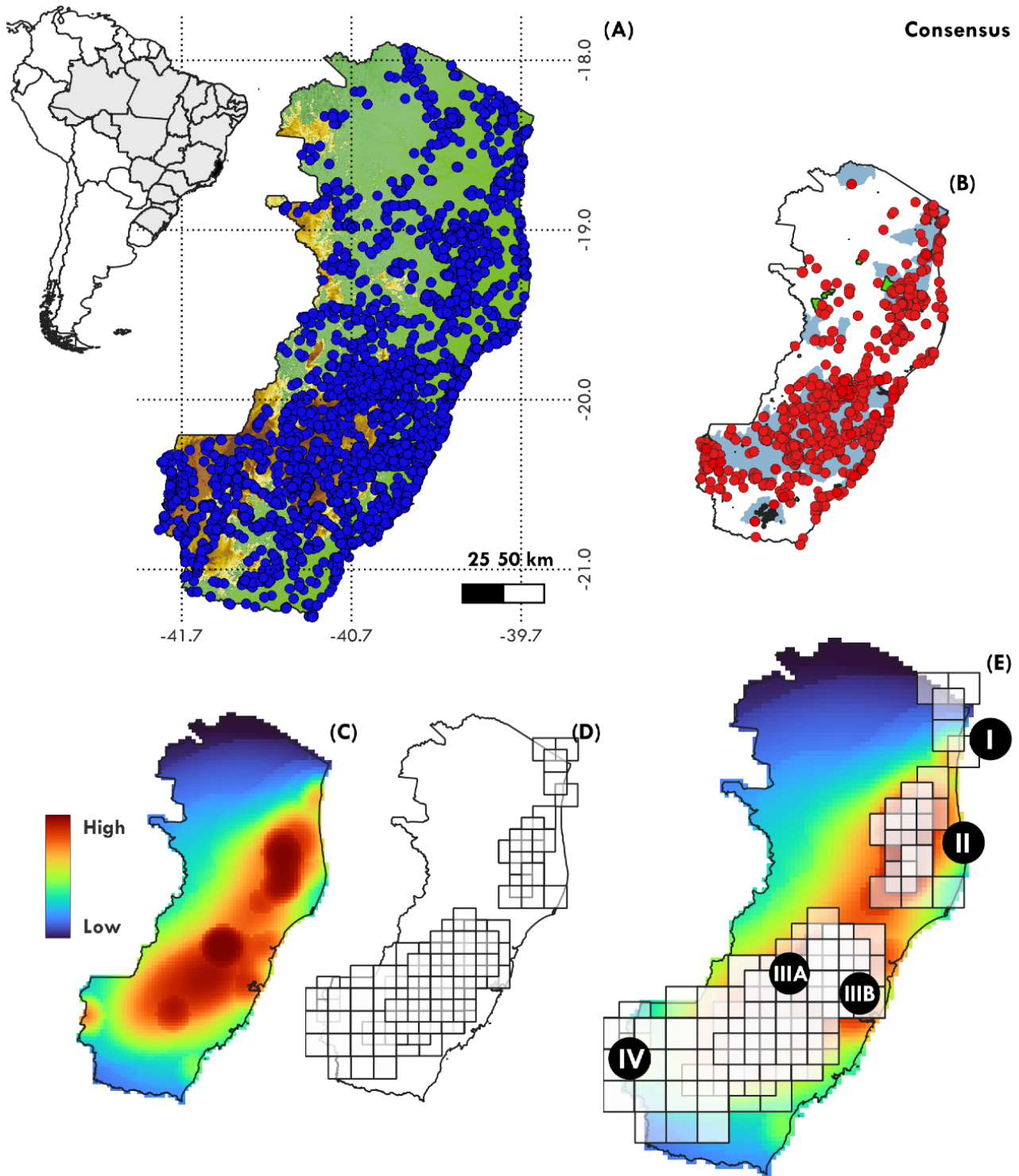


Figure 7. Overlay of areas of endemism for all vertebrate groups in the state of Espírito Santo. (A) Records considered in this study (blue dots); (B) Records of threatened species (red dots), location of protected areas (green) and priority areas for conservation of fauna according to MMA (2018) (light blue); (C) Overlay of GIE outputs; (D) Overlay of NDM outputs; (E) Overlay of the GIE + NDM outputs and suggested priority areas for conservation I (Northeastern Coast), II (Linhares-Sooretama-Lower Doce Complex), III (Central-Southern Highlands and Vitória surroundings) and IV (Caparaó Highlands).

Clelia plumbea (Wied-Neuwied 1820), *Lygophis meridionalis* (Schenkel 1901); dozens of birds including the parrots *Amazona farinosa* (Boddaert 1783) and *Amazona rhodocorytha*, the Mutum-do-Sudeste *Crax blumenbachii* Spix 1825, and the hawk *Harpagus bidentatus* (Latham 1790); the non-flying mammals *Alouatta guariba* (Humboldt 1812), *Chaetomys subspinosus* (Olfers 1818), *Monodelphis iheringi* (Thomas 1888), *Panthera onca* (Linnaeus 1758), *Sapajus robustus* and the bats *Choeroniscus minor* (Peters 1868), *Diaemus youngii* (Jentink 1893), and *Lonchorhina aurita* Tomes 1863 (Moreira et al. 2008, Simon 2009, Mendes et al. 2010, Sarmento-Soares and Martins-Pinheiro 2014, Bérnils et al. 2014, 2019, Chaves et al. 2019, Costa et al. 2019, Ferreira et al. 2019, Fraga et al. 2019, Hostim-Silva et al. 2019, Vela-Ulian et al. 2021, 2024). The primary threat to terrestrial vertebrates in this region stems from the BR-101 highway, which traverses the Reserva Biológica de Sooretama and the Reserva Natural Vale over a 25 km segment. Numerous species, including threatened ones such as the tapir *Tapirus terrestris* (Linnaeus 1758), the jaguar *Panthera onca*, the puma *Puma concolor* (Linnaeus 1771), and the harpy eagle *Harpia harpyja* (Linnaeus 1758), have been documented as roadkill victims (Klippel et al. 2015; Srbek-Araujo et al. 2015; Pagotto 2018; Damásio et al. 2021; Banhos et al. 2021).

III. Central-Southern Highlands and Vitória surroundings: This extensive priority area south of the Rio Doce basin can be divided into two distinct regions: the mountainous region (IIIA), covering the municipalities of Santa Teresa, Santa Maria de Jetibá, Domingos Martins, Santa Leopoldina, Marechal Floriano, Vargem Alta, Castelo, and Alfredo Chaves; and the metropolitan region (IIIB), including Vitória, Vila Velha, Cariacica, Viana, Serra, Fundão, and Aracruz. The primary center of endemism is in Santa Teresa, known as a biodiversity hotspot (Thomaz and Monteiro 1997, Brown and Freitas 2000, Passamani et al. 2000, Simon 2000, Ferreira et al. 2019, Hoppe et al. 2020).

However, the high species richness here may be somewhat skewed due to intensive sampling, being close to the main biodiversity research center of Espírito Santo (Mendes et al. 2010). Protected areas in the region include Reserva Biológica Augusto Ruschi, Reserva Biológica de Duas Bocas, Parque Estadual de Forno Grande, Parque Estadual da Fonte Grande, among others. This area, which includes the region with the highest urban concentration, is heavily impacted by human activities and hosts a considerable number of endangered species records: the freshwater fish *Brycon dulcis*, *Prochilodus vimboides* Kner 1859, and *Trichogenes claviger* de Pinna, Helmer, Britski & Nunes 2010; the amphibians *Arcovomer passarellii* Carvalho 1954, *Brachycephalus alipioi* Pombal & Gasparini 2006, *Crossodactylodes izecksohni* Peixoto 1983, *Dendrophryniscus carvalhoi* Izecksohn 1994, *Ischnocnema colibri* Taucce, Canedo, Parreiras, Drummond, Nogueira-Costa & Haddad 2018, *Ischnocnema epipeda* (Heyer 1984), *Phrynomedusa marginata* (Izecksohn & Cruz 1976), *Proceratophrys phyllostomus* Izecksohn, Cruz & Peixoto 1999, and *Pseudopaludicola restinga* Cardozo, Baldo, Pupin, Gasparini & Haddad 2018; the reptiles *Ameivula nativo*, *Amphisbaena nigricauda* Gans 1966, *Anolis pseudotigrinus* Amaral 1933, *Bothrops bilineatus* (Wied-Neuwied 1821), *Coronelaps lepidus* (Reinhardt 1861), *Dipsas sazimai* Fernandes, Marques & Argôlo 2010, *Hydromedusa maximiliani* (Mikan 1825), and *Tropidophis paucisquamis* Müller 1901; the birds *Carpornis cucullata* (Swainson 1821), *Chamaeza campanisona* (Lichtenstein 1823), *Geotrygon violacea* (Temminck 1809), *Ramphodon naevius* (Dumont 1818), *Saltator similis* d'Orbigny & Lafresnaye 1837, *Tangara cyanoventris* (Vieillot 1819), *Turdus fumigatus* Lichtenstein 1823 and dozens of other species; the flightless mammals *Abrawayaomys ruschii* Cunha & Cruz 1979, *Alouatta guariba*, *Bradypus torquatus* Illiger 1811, *Callicebus personatus* (É. Geoffroy 1812), *Chaetomys subspinosus*, *Marmosops paulensis* (Tate 1931), *Monodelphis scalops* (Thomas 1888), *Rhagomys rufescens* (Thomas 1886), among others; and the

bat *Lonchorhina aurita* (Moreira et al. 2008, Simon 2000, 2009, Mendes et al. 2010, Almeida et al. 2011, Sarmiento-Soares et al. 2018, Bérnils et al. 2019, Chaves et al. 2019, Costa et al. 2019, Ferreira et al. 2019, Fraga et al. 2019, Hostim-Silva et al. 2019).

IV. Caparaó Highlands: This small area in the southwest of the state comprises the Parque Nacional do Caparaó and its surroundings, in the municipalities of Dorés do Rio Preto, Ibitirama, Divino São Lourenço, Guaçuí and Iúna. The region of this protected area is considered extremely important for the conservation of several animal groups, such as mammals, several endemic amphibians and reptiles, and hundreds of bird species (ICMBio 2015). Endangered species in this region include the freshwater fish *Brycon opalinus* (Cuvier 1819), the amphibians *Cycloramphus bandeirensis* Heyer 1983 and *Pseudopaludicola restinga*, the reptile *Caparaonia itaiquara* Rodrigues, Cassimiro, Pavan, Curcio, Kruth Verdade & Machado-Pellegrino 2009, the birds *Buteo nitidus* (Latham 1790), *Drymophila genei* (Filippi 1847), *Microspingus lateralis* (Nordmann 1835), *Saltator similis*, *Scytalopus speluncae* (Ménétries 1835), *Stephanophorus diadematus* (Temminck 1823), *Tangara cyanoventris* and many others; and the non-flying mammals *Delomys altimontanus* Gonçalves & Oliveira 2014, *Monodelphis iheringi*, *Monodelphis pinocchio* Pavan 2015, *Monodelphis scalops*, *Oxymycterus caparaeo* Hershkovitz 1998, and *Tapirus terrestris* (Bérnils et al. 2019, Chaves et al. 2019, Costa et al. 2019, Fraga et al. 2019).

In a previous evaluation, 28 priority conservation areas were identified in Espírito Santo (IPEMA 2005). Since then, only one protected area has been established (Rosa et al. 2019). Therefore, we emphasize the urgent need for new conservation units and measures to mitigate losses of biodiversity and habitat in Espírito Santo. Additionally, attention should be given to the western and northwestern regions,

including the Far North, Northwest, and Polo Colatina micro-regions, primarily in the headwaters of Rio Itaúnas and Rio São Mateus basins. This area is poorly sampled (Fig. 7A-B), particularly for mammals (Moreira et al. 2008, Mendes et al. 2010). There is only one significant conservation unit (Monumento Natural dos Pontões Capixabas), with no priority areas designated by the MMA (2018) and only two identified by IPEMA (2005). Endangered vertebrate species in the region, including amphibians, reptiles, and birds, are largely unprotected.

In this context, National Action Plans (PANs) and Territorial Action Plans (PATs) have been developed to assist in the formulation of conservation policies for threatened species and natural environments, coordinating research and priority actions across different regional scales (Vieira-Guimarães et al. 2024). In Espírito Santo, ongoing action plans include PAN Peixes e Eglas da Mata Atlântica (MMA/ICMBio 2019), focused on freshwater fish and crustaceans in Atlantic Forest rivers; PAN Rivulídeos (MMA/ICMBio 2022a), conserving fish species in the Rivulidae family; PAN Pequenos Mamíferos de Áreas Florestais (MMA/ICMBio 2022b), targeting small mammals in forested areas; PAN Aves da Mata Atlântica (MMA/ICMBio 2023), focusing on endangered birds in the biome; and PAT Capixaba-Gerais (IEF 2021, IEMA 2021), aiming to conserve threatened flora and fauna species in Espírito Santo and Minas Gerais.

In this study, we identified general patterns of richness and diversity across vertebrate endemism areas, outlining four primary regions while noting a gap in northwestern Espírito Santo. Caution is advised, as recognized biodiversity patterns may be influenced by sampling biases, indicating more effective sampling efforts in certain areas. Additionally, factors driving biodiversity fluctuations, such as land use changes, climate variability, and natural resource exploitation, should be considered alongside study findings. Our dataset provides insights into vertebrate

distribution patterns, highlights areas in need of attention, and identifies regions for future surveys. In a multidisciplinary approach, understanding species distribution, habits, and evolutionary histories is crucial for developing conservation policies and guiding future research, particularly in less protected areas of Espírito Santo.

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