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Ranacephala hogei (Mertens, 1967): new occurrence records, natural history, and subsidies for management and conservation status assessment

Clodoaldo Lopes de Assis
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2024

CLODOALDO LOPES DE ASSIS

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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Animal, para obtenção do título de *Doctor Scientiae*.

Orientador: Renato Neves Feio

Coorientadora: Camila Moura Novaes

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“Presuma que a pessoa com quem você está conversando possa saber algo que você não sabe”.

(Jordan Peterson)

RESUMO

ASSIS, Clodoaldo Lopes de, D.Sc., Universidade Federal de Viçosa, julho de 2024. Ranacephala hogei (Mertens, 1967): novos registros de ocorrência, história natural e subsídios para manejo e avaliação do estado de conservação. Orientador: Renato Neves Feio. Coorientadora: Camila Moura Novaes.

Na presente tese apresentamos os resultados de um estudo de longo prazo realizado com o ameaçado cágado *Ranacephala hogei* (Mertens, 1967). Essa espécie é endêmica da Mata Atlântica do Sudeste do Brasil, ocorrendo nos estados de Minas Gerais, Rio de Janeiro e Espírito Santo. Vive nas bacias dos rios Paraíba do Sul, Itapemirim, da Lagoa Feia e do Córrego São Salvador. *Ranacephala hogei* é mundialmente considerada “Criticamente Ameaçada” (CR), sendo o único quelônio brasileiro incluído entre as 25 espécies de tartarugas mais ameaçadas do planeta. Além disso, é a primeira espécie de cágado do Brasil escolhida para um programa de manejo em cativeiro. Todo esse destaque se deve às ameaças impostas a essa espécie, uma vez que sua distribuição se concentra nas regiões mais populosas do país. No entanto, mesmo com todo esse apelo conservacionista, *R. hogei* possui grande carência de estudos, e até mesmo dados básicos são escassos. A falta desses dados prejudica as ações de conservação, compromete as avaliações sobre seu status de conservação e fragiliza a alocação eficiente de recursos para a espécie. Diante desse cenário, realizamos uma pesquisa de longo prazo para atualizar sua área de distribuição e fornecer dados sobre sua história natural, com foco em sua coloração. Iniciamos a coleta de dados em 2016, envolvendo capturas com armadilhas, visitas a coleções científicas, revisão de literatura, ciência cidadã e análises de espectrofotometria. Entre esses métodos, a ciência cidadã se mostrou altamente importante, contribuindo com a grande maioria dos registros. Os resultados ampliaram os pontos de ocorrência conhecidos em 144%, totalizando 45 localidades de ocorrência da espécie. Esses registros expandiram sua distribuição, adicionaram uma nova bacia hidrográfica à sua área de ocorrência e revelaram populações previamente consideradas extintas. Em relação à espectrofotometria, os resultados revelaram que a coloração de *R. hogei*, apesar de chamativa, lhe confere camuflagem no ambiente aquático. A espécie também apresenta diferenças sexuais e ontogenéticas em relação ao brilho, sendo as fêmeas mais brilhantes que os machos. Acreditamos, ainda, que seu comportamento tímido está relacionado às suas cores conspícuas. Por fim, nossos dados trouxeram informações relevantes que contribuirão para futuras avaliações sobre o status de conservação de *R. hogei*, bem como para seu manejo ex situ.

Palavras-chave: Mata Atlântica; Chelidae; Testudines; conservação; distribuição geográfica; coloração

ABSTRACT

ASSIS, Clodoaldo Lopes de, D.Sc., Universidade Federal de Viçosa, July, 2024. Ranacephala hogei (Mertens, 1967): new occurrence records, natural history, and subsidies for management and conservation status assessment . Adviser: Renato Neves Feio. Co-adviser: Camila Moura Novaes.

Here we present the results of a long-term study on the threatened freshwater turtle *Ranacephala hogei* (Mertens, 1967). This species is endemic to the Atlantic Forest of southeastern Brazil and occurs in the states of Minas Gerais, Rio de Janeiro, and Espírito Santo. It inhabits the river basins of the Paraíba do Sul, Itapemirim, Lagoa Feia, and Córrego São Salvador. *Ranacephala hogei* is globally classified as Critically Endangered (CR) and is the only Brazilian chelonian included among the 25 most threatened turtle species worldwide. In addition, it is the first freshwater turtle species in Brazil selected for a captive management program. This prominence reflects the severe threats faced by the species, as its distribution is concentrated in some of the most densely populated regions of the country. Nevertheless, despite its high conservation relevance, *R. hogei* remains poorly studied, and even basic biological data are scarce. The lack of such information hampers conservation actions, compromises assessments of its conservation status, and weakens the efficient allocation of resources directed toward the species. In this context, we conducted a long-term study to update its geographic distribution and provide data on its natural history, with particular emphasis on coloration. Data collection began in 2016 and involved trapping, visits to scientific collections, literature review, citizen science, and spectrophotometric analyses. Among these approaches, citizen science proved to be particularly important, contributing the majority of occurrence records. Our results increased the number of known occurrence localities by 144%, totaling 45 localities for the species. These records expanded its known distribution, added a new river basin to its range, and revealed populations previously considered extinct. Spectrophotometric analyses showed that, despite its conspicuous appearance, the coloration of *R. hogei* provides effective camouflage in aquatic environments. The species also exhibits sexual and ontogenetic differences in brightness, with females being brighter than males. We further suggest that its shy behavior is associated with its conspicuous coloration. Overall, our findings provide valuable information that will contribute to future assessments of the conservation status of *R. hogei* and to the development of effective ex situ management strategies.

Keywords: Atlantic Forest; Chelidae; Testudines; conservation; geographic distribution; coloration

SUMÁRIO

1. INTRODUÇÃO GERAL

As extensas atividades antrópicas têm alterado a dinâmica e o funcionamento dos ecossistemas nas últimas décadas, provocando sérios danos à biodiversidade (Hooper *et al.* 2012). Esses danos impulsionam as atuais taxas de extinção de espécies, que são centenas ou milhares de vezes mais rápidas do que as taxas “normais” prevalecentes nas últimas dezenas de milhões de anos (Sala *et al.* 2000, Ceballos *et al.* 2020). Por exemplo, mais de 400 espécies de vertebrados foram extintas nos últimos 100 anos, extinções que, no curso normal da evolução, teriam levado até 10.000 anos (Ceballos *et al.* 2015). Essas extinções, quando ocorrem no nível populacional, ameaçam diretamente os serviços ecossistêmicos e são um prelúdio para a extinção no nível de espécie (Ceballos & Ehrlich 2002). Além disso, quando uma espécie desaparece, uma grande variedade de características se perde para sempre, desde genes e interações até fenótipos e comportamentos (González-Maya *et al.* 2017). Portanto, considerando a magnitude desse problema, obter informações sobre espécies à beira da extinção é essencial para embasar políticas de conservação mais eficazes e diminuir a atual crise de biodiversidade (Böhm *et al.* 2013, González-del-Pliego *et al.* 2019).

Dos atuais clados de vertebrados, as tartarugas, cágados e jabutis (Ordem Testudines) formam um dos grupos mais ameaçados do mundo. Das 360 espécies modernas, cerca de 60% se encontram em algum grau de ameaça ou já foram extintas (Rhodin *et al.* 2018; TCC 2018). A décima e mais recente extinção ocorreu em 2012, com a morte do último espécime da tartaruga-gigante-de-pinta, *Chelonoidis abingdonii* (Günther, 1877), nas Ilhas Galápagos (Edwards *et al.* 2013, TCC 2018). Outras espécies não são mais encontradas na natureza e existem apenas em cativeiro, como a tartaruga-gigante-de-Yangtze, *Rafetus swinhoei* (Gray, 1873) (TCC 2018; Ennen *et al.* 2020). Agravando esse cenário, mesmo após a mitigação de uma ameaça, a recuperação de populações de tartarugas é lenta devido à longevidade extrema, ao longo tempo de geração, ao atraso na maturidade sexual e à alta sensibilidade à perda de adultos (Brooks *et al.* 1991; Enneson & Litzgus 2009; Spencer *et al.* 2017). Sem surpresa, um estudo recente não encontrou recuperação em uma população de *Chelydra serpentina* (Linnaeus, 1758) que sofreu mortalidade em massa na década de 1980, no Canadá (Keevil *et al.* 2018). Portanto, esses traços característicos dos Testudines podem impedir recuperações populacionais rápidas e levar esse grupo a sofrer declínios lentos e de longo prazo (Lovich *et al.* 2018).

Mesmo estando em uma situação crítica, as tartarugas são frequentemente negligenciadas, pois tradicionalmente são agrupadas com outros “répteis” durante pesquisas (veja Myers *et al.* 2000; Fa & Funk 2007). No entanto, cada táxon incorpora propriedades evolutivas e ecológicas únicas, que criam seus próprios legados de especiação, dispersão e extinção (Holt *et al.* 2013). Para as tartarugas, esses legados só foram rigorosamente estudados recentemente, revelando padrões distintos de diversidade, com regiões de riqueza e endemismo diferentes das de outros grupos de vertebrados (Roll *et al.* 2017; Ennen *et al.* 2020). Esse fato resultou na identificação equivocada de regiões prioritárias para tartarugas no passado (Roll *et al.* 2017). Como resultado, esse grupo ficou ainda mais vulnerável, pois a maioria das prioridades globais de conservação se concentrou em áreas com altos níveis de diversidade e endemismo gerais (Myers *et al.* 2000; Mittermeier *et al.* 2004).

Sem estudos e proteção, os impactos aos quais as tartarugas estão sujeitas são diversos. Como espécies de vida longa e geralmente com dietas amplas, elas bioacumulam altas cargas de contaminantes em locais poluídos, o que pode ter consequências para a reprodução (Bergeron *et al.* 2007, Hopkins *et al.* 2013). A perda e fragmentação de habitats podem diminuir o sucesso das incubações e reduzir a variabilidade genética das populações (Ennen *et al.* 2010, Gallego-García *et al.* 2019). O aumento da temperatura global pode levar a mudanças na proporção entre os sexos em espécies com determinação sexual dependente da temperatura (Schwanz & Janzen 2008). Além de impactos relacionados à degradação ambiental, as tartarugas também estão sujeitas a ameaças exclusivas de determinadas regiões. Na Ásia, por exemplo, a caça de tartarugas para a fabricação de remédios tradicionais, alimentos e para o comércio de animais de estimação é uma ameaça mesmo em áreas protegidas (Gong *et al.* 2017, Sung & Fong 2018). Espécies invasoras, como a raposa-vermelha-europeia (*Vulpes vulpes*), gatos e porcos domésticos também são um problema, pois predam ovos e filhotes (Fordham *et al.* 2008; Dawson *et al.* 2016; Woinarski *et al.* 2018). Por fim, doenças vêm sendo registradas (Johnson *et al.* 2008, Jacobson *et al.* 2014, Sim *et al.* 2015), e uma patologia desconhecida quase levou a tartaruga australiana *Myuchelys georgesi* (Cann, 1997) à extinção em menos de um mês (Spencer *et al.* 2018). De fato, as tartarugas precisam de atenção urgente para evitar extinções catastróficas em um futuro imediato (Lovich *et al.* 2018; Stanford *et al.* 2020).

No Brasil, os Testudines são representados por 34 espécies continentais (representantes das famílias Kinosternidae, Emydidae, Geoemydidae, Testudinidae, Chelidae e Podocnemididae) e cinco espécies marinhas (Cheloniidae e Dermochelyidae), correspondendo a cerca de 10% das espécies mundiais (Guedes *et al.* 2023; Uetz *et al.* 2023). Essa diversidade é bastante representativa, porém ainda pouco conhecida. Por exemplo, um estudo genético com populações de *Phrynops geoffroanus* (Schweigger, 1812) revelou a presença de diferentes linhagens ao longo de sua distribuição, indicando a existência de um complexo de espécies (Carvalho *et al*. 2017). Entre 2020 e 2022, foram descritas duas novas espécies de *Mesoclemmys* exclusivas do Brasil e uma nova espécie de *Chelus* da região amazônica (Vargas-Ramírez *et al*. 2020; Cunha *et al*. 2020, 2022). A história de vida da maioria das tartarugas brasileiras também ainda é pouco estudada. Espécies como *Mesoclemmys perplexa* Bour & Zaher 2005, além de sua descrição, possuem apenas raras informações sobre sua distribuição (Campos *et al*. 2011). Mesmo para espécies descritas há mais tempo, como *Acanthochelys spixii* (Duméril & Bibron, 1835), ainda são necessários estudos básicos sobre sua biologia para subsidiar avaliações de risco de extinção (Vogt *et al*. 2023). Quanto ao status de conservação, em níveis nacional e global, todas as espécies marinhas que ocorrem no Brasil estão ameaçadas, e, das espécies continentais, sete cágados e um jabuti estão classificados em algum grau de ameaça (MMA 2022, IUCN 2024).

Dentre os Testudines ameaçados no Brasil, o cágado-do-paraíba, *Ranacephala hogei* (Mertens, 1967), pertencente à família Chelidae, é a espécie que se encontra em situação mais delicada. Esse cágado é endêmico da Mata Atlântica do sudeste do Brasil e ocorre em áreas de menos de 500 m de altitude na bacia do rio Paraíba do Sul (estados de Minas Gerais e Rio de Janeiro) e na bacia do rio Itapemirim (estado do Espírito Santo) (Mittermeier *et al*. 1980, Rhodin *et al*. 1982). Sua área de distribuição está entre as regiões mais populosas do país, o que gera severas ameaças à sobrevivência da espécie devido ao desmatamento, construção de barragens e poluição causada pela agricultura, resíduos domésticos e industriais (TCC 2018). Esse conjunto de impactos fez com que *R. hogei* fosse classificada como vulnerável (VU) em nível nacional e criticamente ameaçada (CR) em nível global (Drummond *et al*. 2022, MMA 2022), além de incluí-la nas listas vermelhas dos estados onde ocorre (Bergallo *et al*. 2000, COPAM 2010, Fraga *et al*. 2019). Além disso, essa é a única espécie brasileira que está entre os 25 quelônios mais ameaçados do mundo (TCC 2018), tendo sido recentemente escolhida como prioritária para a reprodução em cativeiro no Brasil (AZAB 2018).

Embora *Ranacephala hogei* possua apelo conservacionista nos níveis nacional e internacional, a espécie é pouco estudada, e até mesmo informações básicas são escassas. Esse cágado foi descrito como *Phrynops hogei* em 1967, com base em um único exemplar coletado em uma localidade denominada "rio Pequeno", no sudeste do estado de São Paulo (Mertens, 1967). Mais de uma década após sua descrição, foram apresentados dados sobre sua distribuição com base em oito exemplares de coleções científicas e quatro espécimes vivos (Mittermeier *et al*., 1980). Posteriormente, sua coloração foi descrita com base em adultos, e sua localidadetipo (rio Pequeno) foi contestada devido à possibilidade de erro na descrição geográfica (Rhodin *et al.* 1982). Oito anos depois, sua morfologia cromossômica foi parcialmente caracterizada a partir de um único macho proveniente do rio Paraíba do Sul, no estado do Rio de Janeiro, e seu cariótipo foi definido como 2n=58 e NF=64 (Reed *et al.* 1991). Após esses estudos, as principais informações sobre *R. hogei* vieram de estudos focados em outras espécies ou revisões taxonômicas. Em um desses trabalhos, McCord *et al.* (2001) realizaram uma revisão morfológica de *Phrynops* e concluíram que esse gênero não era monofilético, criando então o gênero monotípico *Ranacephala* para alocar *R. hogei* (anteriormente *Phrynops hogei*). Alguns anos depois, durante a descrição de *Mesoclemmys perplexa*, Bour & Zaher (2005) contestaram esse arranjo taxonômico e extinguiram o gênero *Ranacephala*, transferindo sua única espécie (*R. hogei*) para o gênero *Mesoclemmys*. Mais recentemente, uma filogenia molecular mostrou que *Mesoclemmys* era parafilético, recuperando *M. hogei* como um táxon irmão de *Mesoclemmys* e *Phrynops* (Thomson *et al.* 2021). Esses dados genômicos robustos, aliados aos detalhes morfológicos e osteológicos já disponíveis (McCord *et al.* 2001, Holley *et al.* 2020), deram suporte para restaurar a alocação de *M. hogei* no gênero monotípico *Ranacephala* (TTWG 2021).

Outras informações sobre *Ranacephala hogei* vêm de um monitoramento no rio Carangola, subafluente do rio Paraíba do Sul, em Minas Gerais, que mantém uma subpopulação prósperas da espécie (Drummond *et al.* 2022). Os resultados desse monitoramento indicaram uma maior proporção de adultos e que a maioria dos cágados capturados está perto do tamanho máximo observado para essa espécie, indicando o predomínio da senescência populacional (Moreira 2003, Drummond *et al.* 2022). Além disso, desde 1992, essa subpopulação vinha diminuindo a uma taxa média anual de 16,2%, com extinção local projetada para cerca de sete anos se nada fosse feito, sugerindo que esse declínio poderia estar ocorrendo ao longo de toda a distribuição da espécie (Drummond *et al.* 2022).

Considerando a perspectiva desfavorável, é crucial verificar a concretização dessa projeção para compreender a situação atual da espécie e avaliar com mais precisão o grau de ameaça de *R. hogei*. Assim, esta tese é resultado das pesquisas desenvolvidas ao longo do doutorado do candidato Clodoaldo Lopes de Assis, sob a orientação do Professor Renato Neves Feio no Programa de Pós-Graduação em Biologia Animal do Departamento de Biologia Animal da Universidade Federal de Viçosa. Este trabalho foi realizado em colaboração com Rafael Martins Valadão e Sonia Helena Santesso, analistas ambientais do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). A tese está estruturada em dois capítulos formatados como artigos científicos. O primeiro capítulo apresenta novos registros de ocorrência de *R.*

hogei e foi publicado na revista Anais da Academia Brasileira de Ciências. O segundo capítulo aborda aspectos da história natural da espécie, com foco em sua coloração. Esses dois capítulos trazem dados sobre o cágado-do-paraíba que, em conjunto, fornecem informações valiosas para o manejo e a avaliação do estado atual de conservação da espécie.

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3. CAPÍTULO I

Extensive sampling and citizen science expand the distribution of the threatened freshwater turtle *Ranacephala hogei* (Mertens, 1967)

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ANIMAL SCIENCE

Extensive sampling and citizen science expand the distribution of the threatened freshwater turtle *Ranacephala hogei* (Mertens, 1967)

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Abstract: *Ranacephala hogei* is a South American freshwater turtle considered one of the 25 most endangered chelonian species in the world. Endemic to the Atlantic Forest hotspot of southeastern Brazil, the conservation status of *R. hogei* is subject to continuous assessment at various levels. However, the scarcity of data regarding this species, particularly its geographic range, challenges these evaluations. In an effort to address these gaps, we conducted a comprehensive long-term inventory using different methods to study this species. Our efforts resulted in a 144% increase in documented occurrence points, including a new hydrographic basin and protected areas. By combining historical and current records, we have observed the persistence of *R. hogei* in rural areas, even in locations where its extinction was previously predicted. Consequently, our data significantly contribute to supporting future conservation assessments.

Key words: Atlantic Forest, Chelidae, community science, conservation, geographic range, Testudines.

INTRODUCTION

Geographical distribution data play a vital role in the effective management of threatened species (Saunders et al. 2018). This information is the baseline for various studies in evolution, biogeography, and conservation (Joger et al. 2007, Stuckas et al. 2014, Šarić et al. 2023), supporting assessments of their threat categories (IUCN 2012). This kind of data is particularly crucial today, given the current estimation of extinction rates being 100-1000 times higher than in the past (Lamkin & Miller 2016). Despite advancements, there remains a significant lack of comprehensive knowledge about global, regional, and even local distributions of many species, the 'Wallacean shortfall' (Hortal et al. 2015).

The Wallacean shortfall is essentially alarming for turtles (Testudines), where many species, including critically endangered ones, lack adequate distribution data (Praschag & Singh 2019, Stanford et al. 2020, Fong et al. 2021, Drummond et al. 2022). Furthermore, their long lifespan, delayed sexual maturity, and susceptibility to human activities heighten the impact on this group (Brooks et al. 1991, Enneson & Litzgus 2009, Spencer et al. 2017). Notably, nearly 60% of the roughly 350 modern turtle species face threats or have become extinct (Rhodin et al. 2018). Consequently, turtles are a primary focus in numerous conservation studies, constantly requiring geographic distribution data (Roll et al. 2017, Ennen et al. 2020). Therefore, the success of efforts to reverse declines heavily relies on the quantity and quality of available species distribution data (Whittaker et al. 2005).

One such species, Hoge's Side-necked Turtle, *Ranacephala hogei* (Mertens 1967), is a rare South American Chelidae endemic to the Atlantic Forest hotspot (TCC 2018, TTWG 2021, Drummond et al. 2022). It inhabits three coastal rivers in southeastern Brazil, with most records found in the Paraiba do Sul River basin, an area significantly impacted by human actions (Polaz 2011, TTWG 2021). This freshwater turtle remains poorly studied, and efforts to increase knowledge about its distribution are limited and dispersed (Mittermeier et al. 1980, Rhodin et al. 1982, Melo & Bruno 2016). *Ranacephala hogei* is prominently featured in conservation efforts at different levels. At the regional level, it is listed as Vulnerable, Endangered, and Critically Endangered in the states of Rio de Janeiro, Minas Gerais, and Espírito Santo, respectively (Bergallo et al. 2000, COPAM 2010, Fraga et al. 2019). Additionally, it is part of the National Action Plan for the Conservation of Endangered Aquatic Species in the Paraíba do Sul River Basin (Polaz et al. 2011). On a larger scale, *R. hogei* has been earmarked as a priority species for captive breeding in Brazil (AZAB 2018), and is listed among the 25 most endangered turtle species worldwide (TCC 2018).

Recently, the conservation status of *R. hogei* was reassessed at national and global levels, resulting in contrasting outcomes. In the Brazilian Red List, the species is labeled as Vulnerable, based on its area of occupancy (MMA 2022, Vogt et al. 2023), while the IUCN Red List categorizes it as Critically Endangered due to the decline in a single population (Drummond et al. 2022). Both assessments lack robust distribution data due to incomplete information about the species geographic range and population monitoring within its habitat.

Therefore, we aim to gather information about this imperiled species and aid in planning effective conservation actions, by: i) investigating whether the distribution of *R. hoge*i is confined solely to the river basins cited in the literature; ii) observing whether the species is rare across its range.

MATERIALS AND METHODS

Study sites

Our study area covered the middle and lower sections of the Paraíba do Sul and Itabapoana river basins, as well as the Lagoa Feia and São João river microbasins, in southeastern Brazil. The predominant climates (Köppen system) are Cwa (humid subtropical with dry winters and hot summers) and Aw (tropical with dry winters), with average annual temperatures of 21-25°C respectively, and annual precipitation ranging from 1000-1600 mm (Alvares et al. 2013). This region is within the Atlantic Forest hotspot (Mittermeier et al. 2004) and is mainly covered by Seasonal Semideciduous Forest and Dense Ombrophilous Forest (IBGE 2012). However, this area is one of the most densely populated in Brazil (IBGE 2021), leading to extensive landscape alterations dominated by pastures, farmlands, and urban environments (Joly et al. 2014). Similarly, rivers have been heavily impacted by industrial activities, domestic sewage, mineral exploration, and inappropriate land use (Sousa 2004, Polaz et al. 2011, Gomes et al. 2022).

Field sampling

We obtained distribution data for *Ranacephala hogei* using different methods. Field sampling was conducted in 2016, and subsequentially each year from 2018 to 2023 (collection permits SISBIO 55868, 62603 and 72576). Sampling took place across the river basins of the Paraíba do Sul (30 points), Itabapoana (12 points), São João

(8 points), and Lagoa Feia (2 points) (Figures 1 and 2b). Each sampling ranged from one to three nights. We used 5-50 hoop traps (Lagler 1943), installed on the margins of each sampling site. Hoop traps measured 80-100 cm in length and 40-50 cm in height, with two entrances of 25-30 cm diameter, and 2 cm mesh. Bait was composed of a mixture of meat, liver, canned sardines, cat food, and pineapple. The traps were checked daily, primarily in the early morning. The cumulative effort using this method was 125 sampling nights and 1648 trap/nights (Table I). All captured animals were measured, marked, and released at the same capture location, and this data will be addressed in another study.

Literature review

To ascertain documented records of the species, we searched for peer-reviewed publications using the key words "Phrynops hogei" OR "Mesoclemmys hogei" OR "Ranacephala hogei" in Scopus (https://www.scopus.com/), Web of Science (https://www.webofknowledge.com), and Google Scholar (https://scholar.google.com/) databases in January 2024. Additionally, we did a comprehensive review across all issues of the journal Herpetological Review (https://ssarherps.org/publications/herpetological-review/) and the publications from the Chelonian Research Foundation (https://chelonian.org/crf-publications/).

Figure 1. New recording sites of *Ranacephala hogei*. Minas Gerais: (a) Carangola River, Tombos; (b) Monos River, Recreio. Rio de Janeiro: (c) Negro River, Itaocara. Espírito Santo: (d) Itabapoana River, Mimoso do Sul.

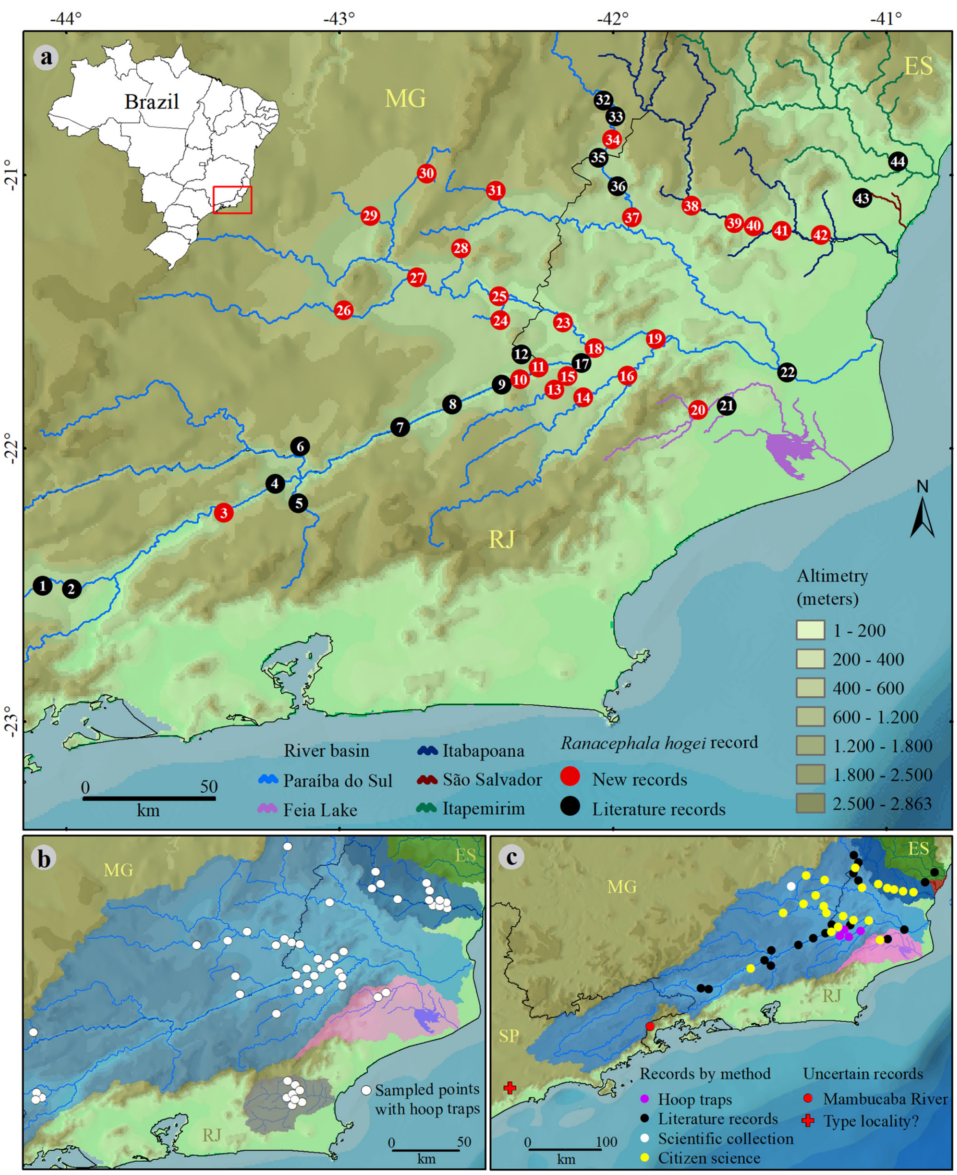

Figure 2. Updated known distribution map of *Ranacephala hogei*. (a) New records and confirmed literature records (locality numbers correspond to Table II). (b) Sampling points with hoop traps. (c) Details of the records by methodology and uncertain records. The river basins in maps b and c correspond to the legend in map a, with the exception of the São João River basin, highlighted in gray in map b. Federative states: MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro, SP = São Paulo.

Table I. Details of sampled areas and capture effort in the *Ranacephala hogei* survey. States: MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro. River basin: PS = Paraíba do Sul, LF = Lagoa Feia, SJ = São João, ITB = Itabapoana. Datum = WGS 84.

Year	Month	Municipality	State	River basin	Site	Coordinates	Effort in trap-night (tn)
2016	Nov.	Aperibé/Cambuci	RJ	PS	Pomba River	-21.6345 -42.0663	10 traps x 1 night (10 tn)
2016	Nov.	Itaocara/Aperibé	RJ	PS	Paraíba do Sul River	-21.6439 -42.0491	30 traps x 1 night (30 tn)
2016	Nov.	Itaocara	RJ	PS	Lowland swamps	-21.6341 -42.0300	7 traps x 2 night (14 tn)
2016	Nov.	Cambuci	RJ	PS	Small stream	-21.5439 -41.9336	10 traps x 2 night (20 tn)

Table I. Continuation.

Year	Month	Municipality	State	River basin	Site	Coordinates	Effort in trap-night (tn)
2016	Nov.	Pirapetinga/Santo Antônio de Pádua	MG/ RJ	PS	Pirapetinga River	-21.7083 -42.2669	38 traps x 3 night (114 tn)
2016	Nov.	Aperibé/Santo Antônio de Pádua	RJ	PS	Small stream	-21.6799 -42.1857	10 traps x 2 night (20 tn)
2016	Nov.	Itaocara/São Sebastião do Alto	RJ	PS	Negro River	-21.7386 -41.9404	30 traps x 2 night (60 tn)
2016	Nov.	Itaocara	RJ	PS	Lake	-21.7320 -41.9443	10 traps x 2 night (20 tn)
2016	Nov.	Itaperuna	RJ	PS	Muriaé River	-21.1974 -42.0317	40 traps x 2 night (80 tn)
2018	Oct.	Campos dos Goytacazes	RJ	LF	Mocotó River	-21.8663 -41.6911	20 traps x 3 night (60 tn)
2018	Oct.	Campos dos Goytacazes	RJ	LF	Imbé River	-21.8646 -41.6787	20 traps x 3 night (60 tn)
2018	Sept.	Itaocara	RJ	PS	Areias Stream	-21.7131 -42.1419	6 traps x 2 night (12 tn)
2018	Sept.	Itaocara	RJ	PS	Areias Stream	-21.7443 -42.1666	13 traps x 1 night (13 tn)
2018	Oct.	Itaocara/São Sebastião do Alto	RJ	PS	Negro River	-21.8162 -42.1053	30 traps x 1 night (30 tn)
2018	Mar.	Além Paraíba	MG	PS	Angú River	-21.7183 -42.6930	6 traps x 3 night (18 tn)
2018	Aug.	Além Paraíba	MG	PS	Angú River	-21.7183 -42.6930	6 traps x 3 night (18 tn)
2018	Mar.	Além Paraíba	MG	PS	Aventureiro River	-21.8423 -42.6633	6 traps x 3 night (18 tn)
2018	Aug.	Além Paraíba	MG	PS	Aventureiro River	-21.8423 -42.6633	6 traps x 3 night (18 tn)
2018	Mar.	São João Nepomuceno/ Descoberto	MG	PS	Novo River	-21.4985 -42.9661	6 traps x 3 night (18 tn)
2018	Aug.	São João Nepomuceno/ Descoberto	MG	PS	Novo River	-21.4985 -42.9661	6 traps x 3 night (18 tn)
2018	Mar.	Santa Rita do Jacutinga	MG	PS	Jacutinga River	-22.1083 -44.1099	8 traps x 3 night (24 tn)
2018	July	Santa Rita do Jacutinga	MG	PS	Jacutinga River	-22.1083 -44.1099	8 traps x 3 night (24 tn)
2018	July	Recreio	MG	PS	Monos Stream	-21.4985 -42.4089	6 traps x 3 night (18 tn)
2018	Dec.	Recreio	MG	PS	Monos Stream	-21.4985 -42.4089	6 traps x 3 night (18 tn)

Table I. Continuation.

Year	Month	Municipality	State	River basin	Site	Coordinates	Effort in trap-night (tn)
2018	July	Palma	MG	PS	Pomba River	-21.4867 -42.2912	6 traps x 3 night (18 tn)
2018	Dec.	Palma	MG	PS	Pomba River	-21.4867 -42.2912	6 traps x 3 night (18 tn)
2018	Sept.	Casimiro de Abreu/ Silva Jardim	RJ	SJ	Aldeia Velha River	-22.5014 -42.2648	10 traps x 3 night (30 tn)
2018	Sept.	Casimiro de Abreu/ Silva Jardim	RJ	SJ	Aldeia Velha River	-22.5280 -42.2616	10 traps x 3 night (30 tn)
2018	Sept.	Silva Jardim	RJ	SJ	São João River	-22.5796 -42.2896	20 traps x 3 night (60 tn)
2018	Sept.	Silva Jardim	RJ	SJ	Lake	-22.5366 -42.2633	5 traps x 1 night (5 tn)
2018	Sept.	Casimiro de Abreu/ Silva Jardim	RJ	SJ	Aldeia Velha River	-22.5281 -42.2607	5 traps x 2 night (10 tn)
2018	Sept.	Silva Jardim	RJ	SJ	Lake/ Lowland swamps	-22.5806 -42.2743	17 traps x 2 night (34 tn)
2018	Sept.	Silva Jardim	RJ	SJ	São João River	-22.5790 -42.2778	6 traps x 2 night (12 tn)
2018	Sept.	Silva Jardim	RJ	SJ	São João River	-22.5621 -42.2723	5 traps x 1 night (5 tn)
2019	Oct.	Itaocara	RJ	PS	Areias Stream	-21.7357 -42.1602	20 traps x 2 night (40 tn)
2019	Oct.	Volta Redonda	RJ	PS	Brandão River	-22.5596 -44.0863	21 traps x 2 night (42 tn)
2019	Oct.	Volta Redonda	RJ	PS	Lake	-22.5484 -44.0784	13 traps x 2 night (26 tn)
2019	Oct.	Volta Redonda	RJ	PS	Brandão River	-22.5598 -44.0869	9 traps x 1 night (9 tn)
2019	July	São Francisco do Glória	MG	PS	Glória River	-20.8065 -42.3265	6 traps x 3 night (18 tn)
2019	Oct.	São Francisco do Glória	MG	PS	Glória River	-20.8065 -42.3265	6 traps x 3 night (18 tn)
2019	June	Santo Antônio de Pádua	MG	PS	Pomba River	-21.4926 -42.2407	6 traps x 3 night (18 tn)
2019	Dec.	Santo Antônio de Pádua	MG	PS	Pomba River	-21.4926 -42.2407	6 traps x 3 night (18 tn)
2020	Nov.	Cataguases/ Leopoldina	MG	PS	Pardo River	-21.4366 -42.6721	17 traps x 1 night (17 tn)
2020	Nov.	Cataguases	MG	PS	Lake	-21.4344 -42.6785	7 traps x 1 night (7 tn)

Table I. Continuation.

Year	Month	Municipality	State	River basin	Site	Coordinates	Effort in trap-night (tn)
2020	Nov.	Cataguases	MG	PS	Cágado Stream	-21.4028 -42.6122	17 traps x 1 night (17 tn)
2020	Jan.	São Francisco do Glória	MG	PS	Glória River	-20.8065 -42.3265	6 traps x 3 night (18 tn)
2021	Oct.	Itaocara	RJ	PS	Paraiba do Sul River	-21.6381 -42.0316	8 traps x 2 night (16 tn)
2022	May	São José dos Calçados/Bom Jesus do Norte	ES	ITB	Calçado River	-21.1041 -41.7179	9 traps x 2 night (18 tn)
2022	May	Bom Jesus do Itabapoana/Bom Jesus do Norte	RJ/ ES	ITB	Itabapoana River	-21.1130 -41.7127	21 traps x 2 night (42 tn)
2022	May	Bom Jesus do Itabapoana/São José dos Calçados	RJ/ ES	ITB	Itabapoana River	-21.0313 -41.7257	20 traps x 2 night (40 tn)
2022	May	Bom Jesus do Itabapoana/Mimoso do Sul	RJ/ ES	ITB	Itabapoana River	-21.1776 -41.5556	50 traps x 2 night (100 tn)
2022	Apr.	Itaocara	RJ	PS	Lake	-21.7325 -41.9437	3 traps x 1 night (3 tn)
2022	May	Cantagalo	RJ	PS	Negro River	-21.9788 -42.4056	20 traps x 1 night (20 tn)
2023	May	Mimoso do Sul/ Campos dos Goytacazes	ES/ RJ	ITB	Itabapoana River	-21.2217 -41.3086	20 traps x 2 night (40 tn)
2023	May	Mimoso do Sul	ES	ITB	Muqui do Sul River	-21.1814 -41.3351	20 traps x 2 night (40 tn)
2023	May	Mimoso do Sul	ES	ITB	Lowland swamps	-21.1808 -41.3362	10 traps x 1 night (10 tn)
2023	May	Mimoso do Sul	ES	ITB	Muqui do Sul River	-21.0889 -41.3442	20 traps x 3 night (60 tn)
2023	May	Mimoso do Sul/ São Francisco do Itabapoana	ES/ RJ	ITB	Itabapoana River	-21.2254 -41.2330	23 traps x 1 night (23 tn)
2023	May	Mimoso do Sul	ES	ITB	Drainage channel	-21.2243 -41.2325	14 traps x 1 night (14 tn)
2023	May	Mimoso do Sul	ES	ITB	Lowland swamps	-21.2244 -41.2330	9 traps x 1 night (9 tn)
2023	May	Mimoso do Sul	ES	ITB	Lowland swamps	-21.2198 -41.2326	8 traps x 1 night (8 tn)

Scientific collections

We examined specimens from the following natural history collections in Brazil: Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa, Minas Gerais, (MZUFV); Museu de Zoologia Newton Baião de Azevedo, Universidade do Estado de Minas Gerais, Carangola, Minas Gerais, (MZNB); Coleção de Répteis do Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (UFMG-REP); Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, (MNRJ), Museu de Zoologia da Universidade de São Paulo, São Paulo, (MZUSP), and Museu de Zoologia da Universidade Estadual de Campinas (ZUEC-REP).

Citizen science

During fieldwork, we collected data through citizen science, with volunteers assisting in data collection (Cohn 2008), a method widely used in biological monitoring (Cunha et al. 2017). Engagements were made with fishermen, local residents, environmental police, and other researchers operating in our study areas. We explained the objectives of our study, showing images of *R. hogei*, and left contact details (cellphone number) for communication in case of potential sightings of the species. We treated citizen science records as incidental observations, as we did not recommend specific efforts toward capturing the turtles. We only included records that allowed unequivocal species identification (supported by photographic evidence) and provided the precise location of the record.

Presence in protected areas

To confirm the presence of *R. hogei* in protected areas, we plotted records using Arcmap (v.10.8 ESRI) against a map displaying Brazil's protected areas, (CNUC 2023). We consider protected areas as territorial spaces with significant natural characteristics wherein protection guarantees are applied based on the National System of Nature Conservation Units (Brasil 2000).

RESULTS

During our field samplings, we successfully captured 24 specimens of *R. hogei* in four previously unrecorded localities. Our bibliographic review contributed with 20 occurrence points for this freshwater turtle species, two of these (type locality in São Paulo and the Mambucaba River micro-basin in Paraty, Rio de Janeiro) are uncertain (Figure 2c). Consulting with curators and examining specimens in collections revealed an additional locality for the species. Citizen science efforts provided evidence of 49 specimens from 21 new localities, expanding our geographic reach to areas not sampled by our team (Figure 2c). Notably, four protected areas have records of *R. hogei*: "Refúgio de Vida Silvestre Estadual do Médio Paraíba" and "Área de Proteção Ambiental do Triunfo" in the state of Rio de Janeiro; "Estação Ecológica de Água Limpa" in the state of Minas Gerais; and "Área de Proteção Ambiental Guanandy", in the state of Espírito Santo.

Collectively, these combined efforts resulted in 44 confirmed occurrence points for *R. hogei*, 26 of these being new records (Figure 3). These confirmed occurrences are distributed across the Brazilian states of Minas Gerais, Rio de Janeiro, and Espírito Santo, spanning five hydrographic basins: Paraíba do Sul River (n=35), Itabapoana River (n=5), Itapemirim River (n=1), São Salvador Stream (1), and Lagoa Feia (n=2) (Table II). Regarding the new records, most (80%) were observed in the Paraíba do Sul River basin, primarily in small tributaries close to the main river and within the sub-basins of the Pomba and Muriaé rivers inland. The elevation of these documented points ranged from four

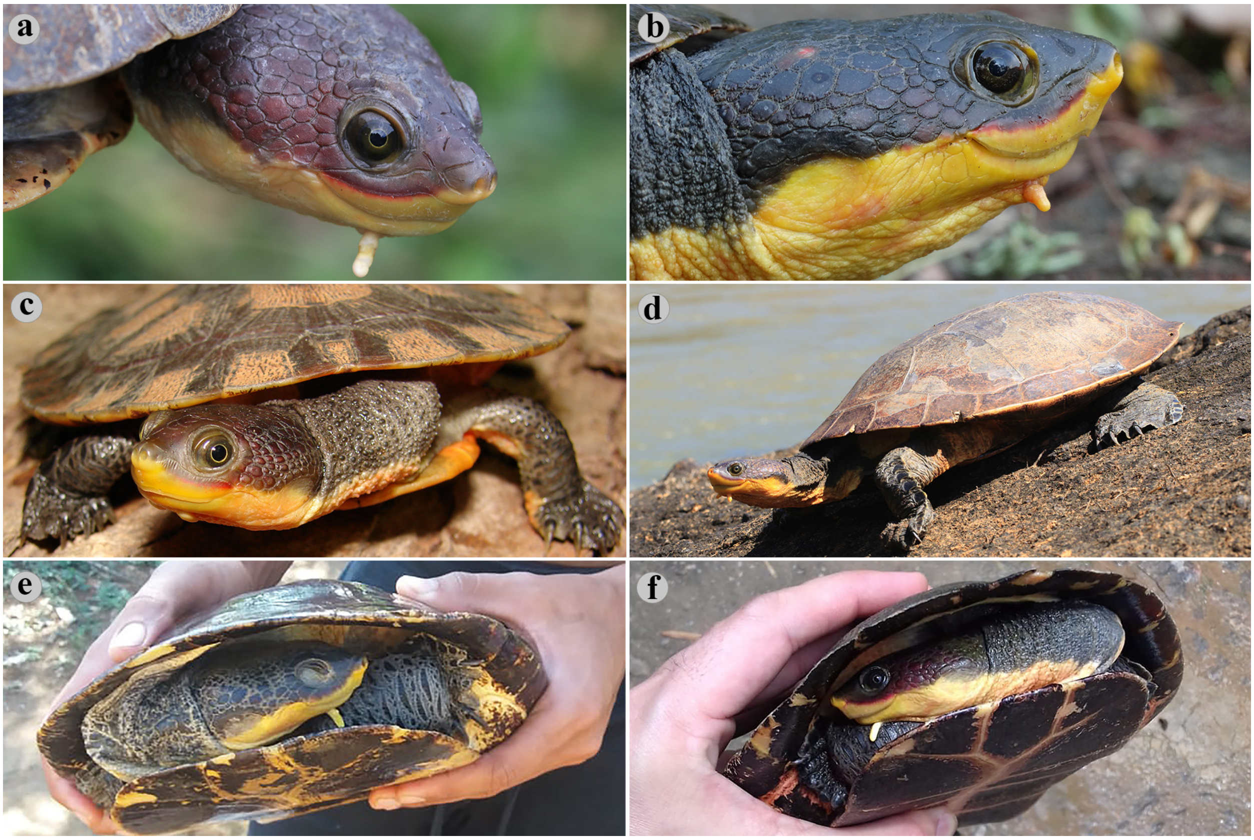

Figure 3. Specimens of *Ranacephala hogei* recorded during our study. Rio de Janeiro/Espírito Santo: (a) juvenile male from the Itabapoana River, Bom Jesus do Itabapoana/Mimoso do Sul. Rio de Janeiro: (b) adult male from the Negro River, Itaocara/São Sebastião do Alto. Minas Gerais: (c) juvenile (indeterminate sex) from the Pomba River, Cataguases; (d) adult male from the Carangola River, Tombos; (e) adult female from the Pomba River, Laranjal/Recreio; (f) juvenile male from the Monos River, Recreio.

(Itapemirim river), to 400 meters above sea level (Carangola river), with an average of 162 meters (± 123).

DISCUSSION

Here, we present a comprehensive study using various methods to update the known geographic distribution of the threatened freshwater turtle *Ranacephala hogei*. Compared to the available literature, our efforts increased the occurrence points of this species by 144%. Notably, our research extended the known range of *R. hogei* westward in the state of Minas Gerais, documenting the species in the headwaters of the Pomba River and the Muriaé River sub-basin, both tributaries of the Paraíba do Sul River. Of particular interest are points 29 and 30 (Figure 2a) in the tributaries of the Pomba River, approximately 176 km from the coast, the most inland records for *R. hogei*. Equally important is the discovery of five *R. hogei* specimens in the Itabapoana River (Figure 2a, points 38 to 42), respectively 23 and 21 km from the nearest records in the Paraíba do Sul River and São Salvador Stream basins. This finding adds a new hydrographic basin (Itabapoana River) where *R. hogei* occurs.

Two records of *R. hogei* are considered uncertain (TTWG 2021) and have been omitted from our distribution map (Figure 2c). The first is the type locality at "Rio Pequeno", a small

Table II. Distribution records of *Ranacephala hogei*. States: MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro. River basin: PS = Paraíba do Sul, LF = Lagoa Feia, ITB = Itabapoana, SS = São Salvador, ITP = Itapemirim. Source: 1 = Mittermeier et al. (1980), 2 = Rhodin et al. (1982), 3 = TTWG (2021), 4 = Gomes et al. (2023). Datum = WGS 84.

Map point	Municipality	State	River basin	Site	Coordinates	Elev. (meters)	Registration method	Source
1	Volta Redonda	RJ	PS	Paraíba do Sul River	-22.5029 -44.0860	370	–	3
2	Pinheiral/ Barra do Piraí	RJ	PS	Paraíba do Sul River	-22.5156 -43.9791	362	–	1, 2, 3
3	Vassouras	RJ	PS	Paraíba do Sul River	-22.2365 -43.4230	292	Citizen science. 1 captured by a local resident.	New record
4	Três Rios	RJ	PS	Paraíba do Sul River	-22.1302 -43.2350	275	–	1, 2, 3
5	Areal	RJ	PS	Preto River	-22.2016 -43.1505	332		4
6	Chiador/Santana do Deserto	MG	PS	Cágado River	-21.9941 -43.1438	299	–	3
7	Além Paraíba/ Sapucaia	MG/RJ	PS	Paraíba do Sul River	-21.9224 -42.7781	143	–	1, 2, 3
8	Volta Grande/ Carmo	MG/RJ	PS	Paraíba do Sul River	-21.8372 -42.5817	103	–	3
9	Estrela Dalva/ Cantagalo	MG/RJ	PS	Paraíba do Sul River	-21.7624 -42.3992	93	–	3
10	Pirapetinga/ Canta Galo	MG/RJ	PS	Paraíba do Sul River	-21.7481 -42.3393	85	Citizen science. 1 captured by a local fisherman.	New record
11	Pirapetinga/ Santo Antônio de Pádua	MG/RJ	PS	Pirapetinga River	-21.7045 -42.2713	81	Citizen science. 1 visualized by a researcher.	New record
12	Pirapetinga/ Santo Antônio de Pádua	MG/RJ	PS	Pirapetinga River	-21.6914 -42.2659	94	–	3
13	Itaocara	RJ	PS	Areias Stream	-21.7441 -42.1664	75	Freshwater turtle surveys. 3 captured by hoop-net trap.	New record
14	Itaocara/São Sebastião do Alto	RJ	PS	Negro River	-21.8144 -42.1074	108	Freshwater turtle surveys. 1 captured by hoop-net trap.	New record

Table II. Continuation.

Map point	Municipality	State	River basin	Site	Coordinates	Elev. (meters)	Registration method	Source
15	Itaocara	RJ	PS	Areias Stream	-21.7127 -42.1437	75	Freshwater turtle surveys. 1 captured by hoop-net trap.	New record
16	Itaocara/São Sebastião do Alto	RJ	PS	Negro River	-21.7347 -41.9464	60	Freshwater turtle surveys. 19 captured by hoop-net trap.	New record
17	Itaocara/Aperibé	RJ	PS	Paraíba do Sul River	-21.6885 -42.1142	60	–	3
18	Aperibé/Cambuci	RJ	PS	Pomba River	-21.6346 -42.0666	57	Citizen science. 1 captured by a researcher.	New record
19	São Fidelis	RJ	PS	Paraíba do Sul River	-21.6002 -41.8424	30	Citizen science. 1 captured by a local fisherman.	New record
20	Campos dos Goitacazes	RJ	LF	Mocotó River	-21.8601 -41.6873	15	Citizen science. 1 captured by a researcher.	New record
21	Campos dos Goytacazes	RJ	LF	Urubú River	-21.8451 -41.5833	9	–	3
22	Campos dos Goytacazes	RJ	PS	Paraíba do Sul River	-21.7223 -41.3616	5	–	1, 2, 3
23	Santo Antônio de Pádua	RJ	PS	Pomba River	-21.5403 -42.1820	90	Citizen science. 1 captured by a local fisherman.	New record
24	Recreio	MG	PS	Monos Stream	-21.4985 -42.4089	160	Citizen science. 1 captured by a local fisherman.	New record
25	Laranjal/ Recreio	MG	PS	Pomba River	-21.4444 -42.4159	145	Citizen science. 1 captured by a local fisherman.	New record
26	Descoberto/ São João Nepomuceno	MG	PS	Novo River	-21.4958 -42.9845	340	Citizen science. 1 captured by a local resident.	New record
27	Cataguases	MG	PS	Small tributary of the Pomba River	-21.3748 -42.7171	263	Citizen science. 1 captured by a researcher.	New record
28	Santana de Cataguases	MG	PS	Fumaça Stream	-21.2609 -42.5497	280	Citizen science. 1 collected by the Environmental Police.	New record

Table II. Continuation.

Map point	Municipality	State	River basin	Site	Coordinates	Elev. (meters)	Registration method	Source
29	Ubá	MG	PS	Ubá Stream	-21.1387 -42.8798	300	Deposited in scientific collection (MNRJ 4803)	New record
30	Guiricema	MG	PS	Bagres Stream	-20.9951 -42.6809	349	Citizen science. 1 captured by a local resident.	New record
31	Muriaé	MG	PS	Preto River	-21.0578 -42.4286	230	Citizen science. 1 visualized by a researcher.	New record
32	Carangola	MG	PS	Carangola River	-20.7449 -42.0111	400	–	3
33	Faria Lemos	MG	PS	Carangola River	-20.8224 -42.0126	327	–	3
34	Tombos	MG	PS	Carangola River	-20.8519 -41.9999	324	Citizen science. 28 captured by a local fisherman.	New record
35	Tombos	MG	PS	Carangola River	-20.9165 -42.0338	220	–	3
36	Natividade	RJ	PS	Carangola River	-21.0416 -42.9829	187	–	3
37	Natividade/ Itaperuna	RJ	PS	Carangola River	-21.1561 -41.9297	130	Citizen science. 1 captured by a local fisherman.	New record
38	Bom Jesus do Itabapoana/Bom Jesus do Norte	RJ/ES	ITB	Itabapoana River	-21.1126 -41.7124	111	Citizen science. 1 captured by a researcher.	New record
39	Bom Jesus do Itabapoana/ Mimoso do Sul	RJ/ES	ITB	Itabapoana River	-21.1772 -41.5546	69	Citizen science. 1 captured by a local fisherman.	New record
40	Bom Jesus do Itabapoana/ Mimoso do Sul	RJ/ES	ITB	Itabapoana River	-21.1826 -41.4996	61	Citizen science. 1 captured by a researcher.	New record
41	Campos dos Goitacazes	RJ	ITB	Itabapoana River	-21.2048 -41.3816	62	Citizen science. 1 captured by a local fisherman.	New record
42	Mimoso do Sul	ES	ITB	Lowland swamps	-21.2193 -41.2391	15	Citizen science. 1 captured by a researcher.	New record
43	Presidente Kennedy	ES	SS	?	-21.0850 -41.0863	52	–	1, 3
44	Itapemirim	ES	ITP	Itapemirim River	-20.9507 -40.9562	4	–	1, 3

tributary of the Tietê River, near the municipality of São Paulo (Mertens 1967). Rhodin et al. (1982) questioned the validity of this record due to the uncertain origin of the holotype, that had been kept not tagged in a serpentarium at the Instituto Butantan. Furthermore, “Rio Pequeno” is at an elevation above 500 meters, while the species inhabits areas with an average elevation of 163 meters, and the nearest confirmed record of *R. hogei* is more than 300 km away from this location. The second uncertain record is from Serra do Mar, in the Mambucaba River micro-basin, state of Rio de Janeiro (TTWG 2021), approximately 80 km from the nearest occurrence of *R. hogei* in the Paraíba do Sul River. Despite the proximity, the record from Serra do Mar is at 1180 m elevation. Even though we did not sample these two areas of uncertain records, we support the hypothesis of the absence of *R. hogei* there.

Moreover, we refined the accuracy of the occurrence points of *R. hogei* in the Itapemirim River basin. Three points of occurrence of the species have been recorded in the state of Espírito Santo, two in the Itapemirim river and one further south in the microbasin of the São Salvador Stream (TTWG 2021). However, a discrepancy arose as these records referred to specimens mentioned in Mittermeier et al. (1980) (A.G.J. Rhodin, personal communication). These authors recorded three live specimens near the mouth of the Itapemirim River, two of which were kept at the Ataliba Farm (currently Usina Paineiras, coordinates adjusted by us), east of the BR-101 highway, and one in the region of the São Salvador Stream. The locality plotted by TTWG (2021) at the mouth of the Rio Itapemirim appears to have been erroneously mapped and is not considered here.

In examining historical records, we found evidence of *Ranacephala hogei* persisting in rural landscapes. Specimens collected in 1952 (MZUSP 2683) and 1984 (MNRJ 10058) in the municipality of Pinheiral, and 1990 (ZUEC-REP 1167) in the municipality of Itaocara, all within the Paraíba do Sul River basin, are housed in scientific collections. In Pinheiral, a study conducted in 2015 recorded 30 observations and captured 18 adults and juveniles (Melo & Bruno 2016), while our study in Itaocara resulted in 24 captures of adults and juveniles. This confirms that the species persists in these impacted areas after 63 and 28 years, respectively. Building on this finding, we highlight an intriguing aspect related to a population of *R. hogei* from the Carangola River, a sub-tributary of the Paraíba do Sul River. Prior research in this area suggested an average annual rate of decline rate of 16.2% since 1992, projecting local extinction within less than seven years (Drummond et al. 2022). However, our results revealed casual sightings of 30 specimens (9 juveniles and 21 adults) caught by fishermen between 2021 and 2023 in the same area. This demonstrates that 31 years after the forecasted local extinction, the species not only endures but also reproduces in the area. This resilience might be due to the species adaptability to habitat changes, contrasting with turtles having specialized habitat needs and specific diets, which render them more susceptible to environmental shifts (Burbidge 1981, Regolin et al. 2023). Indeed, the life history of *Ranacephala hogei* remains unknown, but these findings suggest a certain resilience to anthropogenic changes, potentially owing to a broader ecological niche, allowing for greater adaptability in diet, habitat use, and environmental changes (Fachin-Teran et al. 1995, Souza & Abe 2000, Stokeld et al. 2014).

We underscore the critical role of citizen science in updating the distribution range of *R. hogei*, by adding 21 new records (more that any methods used by this study). This method expanded our geographic reach and revealed records beyond our survey areas, including the most inland records of the species and records within the Itabapoana River, where we extensively sampled (384 traps-night) but had negative results. Citizen science has already provided important data on threatened turtles such as *Macrochelys temminckii* in the United States (Gordon et al. 2023) and *Indotestudo elongata* in Bhutan (Wangyal et al. 2022). However, one particularly impressive case occurred in northern Vietnam, where a field study involving local residents and hunters resulted in the documentation of nine threatened species (Thong et al. 2020, IUCN 2023), with a prevalence of *Cuora galbinifrons*, listed among the 50 most endangered turtle species worldwide (TCC 2018, Thong et al. 2020). Considering the pivotal role of citizen science in studies related to the conservation of endangered turtles (Anadón et al. 2009, Thong et al. 2020, Cross et al. 2021, Gordon et al. 2023), we emphasize the importance of public participation in the future monitoring of *R. hogei*.

The presence of *R. hogei* in protected areas enhances the significance of these locations, given the species' presence in red lists (MMA 2022, Drummond et al. 2022). Of the four protected areas with the species confirmed occurrences, the "Refúgio de Vida Silvestre do Médio Paraíba" is a strictly protected area, corresponding to IUCN category III (Rylands & Brandon 2005). Covering about 111 km^2, it extends along the banks of the Paraiba do Sul River for approximately 186 km in the state of Rio de Janeiro (CNUC 2023). The other protected area with confirmed presence of *R. hogei* is the "Estação Ecológica de Água Limpa", corresponding to IUCN category Ia (Rylands & Brandon 2005). This area consists of a small remnant of native forest with 0.71 km^2, near the Pomba River in the state of Minas Gerais (CNUC 2023). Unlike other protected areas with records of *R. hogei*, which fall into categories with fewer restrictions (IUCN category V), these two areas are under strict protection (Brasil 2000, Rylands & Brandon 2005). Consequently, they contribute more effectively to the preservation of the habitats of this freshwater turtle. However, the effectiveness of this protection could be improved with specific programs and actions tailored to this species. Unfortunately, both protected areas lack management plans (CNUC 2023), crucial for implementing conservation strategies (Thomas & Middleton 2003). Urgent elaboration of these plans is necessary to direct focused actions for the protection of *R. hogei*.

In conclusion, our findings offer optimism regarding the conservation status of *R. hogei*, providing valuable information for future assessments, conservation, and management of this species. The integration of traditional inventory methods with citizen science has revealed a larger distribution range for *R. hogei* than previously assumed, including new hydrographic basins. Moreover, the species might not be as rare as reported in the literature; its perceived rarity may be associated with the absence of specific and extensive sampling efforts. We have also observed that this species survives and reproduces in areas where local extinction was previously predicted. Hence, although the limits of its persistence remain unknown, our findings suggest that, with appropriate conservation efforts, *R. hogei* can thrive in human-dominated rural landscapes. Future research should focus on monitoring the species and investigating gene flow between its habitats. Furthermore, efforts in environmental education should target fishermen and riverside dwellers to underscore the importance of preserving this freshwater turtle.

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4. CAPÍTULO II

Rediscovery of the endangered Hoge's side-necked turtle *Ranacephala hogei* in the Itapemirim River, south-eastern Brazil

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Rediscovery of the endangered Hoge's side-necked turtle *Ranacephala hogei* in the Itapemirim River, south-eastern Brazil

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Hoge's side-necked turtle *Ranacephala hogei* (Mertens, 1967) is one of the most threatened freshwater turtles in South America. This species, belonging to the family Chelidae, is endemic to the Atlantic Forest of south-eastern Brazil (Mittermeier et al., 1980; Assis et al., 2024) and is listed as Critically Endangered on the IUCN Red List and Vulnerable on the Brazilian Red List (Drummond et al., 2022; MMA, 2022). Unsurprisingly, *R. hogei* also appears on the Red Lists of the Brazilian states where it occurs, being assessed as Vulnerable, Endangered and Critically Endangered in Rio de Janeiro, Minas Gerais, and Espírito Santo, respectively (Bergallo et al., 2000; COPAM, 2010; Espírito Santo, 2022). Given this scenario, *R. hogei* is the only Brazilian freshwater turtle listed among the world's 25 most threatened chelonians (Turtle Conservation Coalition, 2025).

Mapping the areas where *R. hogei* is present is crucial for conservation efforts, as the rivers inhabited by this species are located in a region of intense human activity. The species is found at an average altitude of 162 m within the drainage basins of the Paraíba do Sul, Itabapoana and Itapemirim

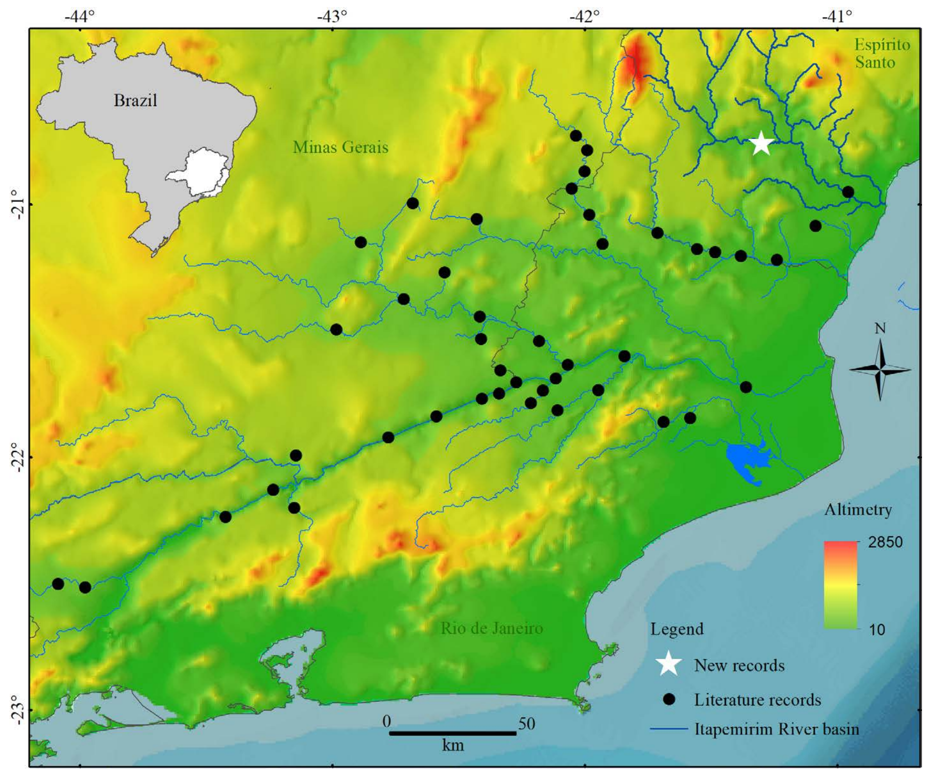

Figure 1. New record (white star) of *Ranacephala hogei* in the Itapemirim River, Espírito Santo state, south-eastern Brazil

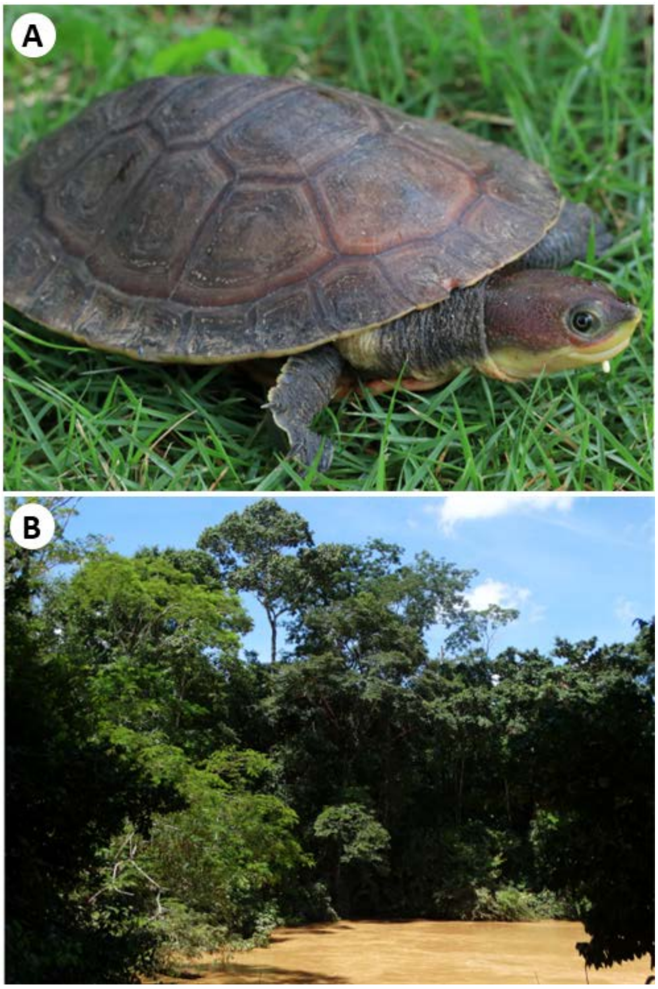

Figure 2. A. Juvenile *Ranacephala hogei* captured in the Itapemirim River, Cachoeiro de Itapemirim municipality, Espírito Santo state, south-eastern Brazil, **B.** Riparian forest environment of the captured turtle

Rivers, as well as in the Lagoa Feia basin and the microbasin of Córrego São Salvador (Mittermeier et al., 1980; Assis et al., 2024). Its distribution area overlaps with some of the most densely populated regions of the country, posing severe threats to its survival due to deforestation, dam construction and pollution (Turtle Conservation Coalition, 2018). Despite these threats, the species persists, with recent records in all drainages where it occurs, except in the Itapemirim River basin (Assis et al., 2024). In this context, we report a new and recent occurrence of *R. hogei* in the Itapemirim River, in the surroundings of an important sustainable-use protected area.

To strengthen our survey effort, we took advantage of local ecological knowledge by holding dialogues with riverside residents and local fishermen, which resulted in the identification of certain sites as potentially harbouring the species. We added these sites to the sampling design of the present study and for five nights (27–31 March 2024), we surveyed (licence Sisbio 55868, 72576) the surroundings of the Floresta Nacional de Pacotuba (Flona de Pacotuba, 20° 44'31" S, 41° 17'37" W, 95 m a.s.l.), in the south of Espírito Santo state, south-eastern Brazil (Fig. 1) for turtles. The Flona de Pacotuba is a sustainable-use protected area corresponding to IUCN category Ia (Rylands & Brandon, 2005) and encompasses 449 hectares within an Atlantic Forest hotspot (Mittermeier et al., 2004). It is located between the Bahia coastal forests and Bahia interior forests ecoregions (Dinerstein et al., 2017). The predominant climate in the region is humid subtropical with dry winters and hot summers (Cwa, Köppen classification), with rainfall reaching a peak November to January, and the peak of the dry season July to September (Alvares et al., 2013; SEPLAN, 1999). The average minimum temperature in the coldest month and the hottest month ranges from 11.8 °C to 18 °C and 30.7 °C to 34 °C, respectively (SEPLAN, 1999). The Flona de Pacotuba is bordered by the Itapemirim River, maintaining a strip of forest along its banks.

We installed 67 hoop traps (Lagler, 1943) in the surroundings of the Flona de Pacotuba, distributing them along the Itapemirim River (50 traps), in a marginal pond adjacent to the river within the forest (3 traps), in a swamp area at the forest edge (4 traps), and in a small tributary of the Itapemirim River (10 traps). The total sampling effort was 176 trap-nights. The traps measured 80 to 100 cm in length and 40 to 50 cm in height, with two entrances of 25 to 30 cm in diameter and a 2 cm mesh. The traps were baited with a mixture of meat, liver, tinned sardines, cat food, and pineapple, and were checked daily in the morning. We identified the specimens based on their morphological and chromatic characteristics following Rhodin et al. (1982). All captured animals were measured, marked and released at the original capture site.

We recorded five specimens of *R. hogei*, of which two juveniles (Fig. 2A) and two adult females were captured in an area with riparian vegetation in the Itapemirim River (Fig. 2B), and one juvenile in a marginal lagoon within the forest, approximately 100 m from the river. The juveniles had straight carapace lengths (CL) of 105.4, 117.8 and 126.6 mm, whilst the adult females had CL of 325.9 and 330.5 mm. Through manual palpation of the inguinal region, we detected the presence of calcified eggs in the larger female.

In Espírito Santo state, *R. hogei* is restricted to the Itapemirim and Itabapoana Rivers, and the São Salvador stream microbasin (Assis et al., 2024). In the Itapemirim River, the only record of this freshwater turtle was made in 1980, at Fazenda Ataliba (currently Usina Paineiras), approximately 16 km from its mouth (Mittermeier et al., 1980; Assis et al., 2024). Our record, therefore, adds a new area of occupancy for *R. hogei* in the Itapemirim River, approximately 66 km upstream from Fazenda Ataliba, and rediscovers the species in this river after 44 years. Our observation of a female with calcified eggs in late March suggests that *R. hogei* follows the same pattern as other South American Chelidae, nesting from the end of the rainy season (Cintra & Yamashita, 1989; Souza & Abe, 2001). Furthermore, the record of a juvenile in a marginal lagoon within the forest suggests that *R. hogei* uses this type of habitat during this stage of life. Habitats such as small lakes and streams provide safer conditions for the development of hatchlings and juveniles (Guix et al., 1992; Souza & Abe, 1997).

Additionally, records of females (including one with eggs) and juveniles of *R. hogei* in the vicinity of the Flona de Pacotuba suggest that the species uses this protected area for reproduction. This protected area harbours forest ecosystems that are currently rare along the banks of the Itapemirim River (Pirovani et al., 2014), including riparian forests, small streams and marginal lagoons. Consequently, the Flona de Pacotuba can be considered a strategic area for the conservation of *R. hogei* within the Itapemirim River basin. Acknowledging this context, we will initiate long-term monitoring of this freshwater turtle in the region of the present record, in collaboration with the managers of the Flona de Pacotuba and the surrounding communities.

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5. CAPÍTULO III

Hatchling color and spectral reflectance of the threatened freshwater turtle *Ranacephala hogei* (Mertens, 1967)

4.1. Abstract

While other vertebrate classes have been more extensively studied about their coloration, turtles are still relatively unexplored in this aspect. However, turtle coloration is fundamental throughout their life cycle. *Ranacephala hogei*, one of the most endangered turtles in the world, exhibits a conspicuous color pattern that remains quite consistent regardless of sex, making it an ideal model for color-related studies. Thus, our objective was to describe for the first time the coloration of hatchlings of this species and to characterize the color pattern of adults and juveniles using visible spectrum (400–750 nm) spectrophotometry techniques. The results showed that hatchlings follow the same color pattern as adults, with few variations. In contrast, spectrophotometry revealed sexual and ontogenetic dichromatism in brightness, showing that females are brighter than males and adults are brighter than juveniles. The data on head coloration were especially interesting, indicating a color and reflection pattern that facilitates heat absorption. The color pattern observed in *R. hogei* is consistent with the species' behavior and habitat preferences, and may be associated with strategies for aquatic camouflage, intraspecific communication, and thermoregulation. These findings not only enhance our understanding of chromatic influences in the species but also provide valuable information for the management and conservation of this turtle threatened with extinction.

Keywords. Testudines, Chelidae, Chromatic adaptation, Natural history, Conservation

4.2. Introduction

Color patterns in turtles may be involved in a range of processes, including intra and interspecific communication as well as thermoregulation. In interspecific contexts, turtles with colors that obscure their outlines or mimic the color of their environment benefit from camouflage, which reduces detection by both predators and prey (Ross & Lovich 1992; Ewert *et al*. 2006; Xiao *et al*. 2016). Additionally, hatchlings of various freshwater turtle species exhibit bright colors on the ventral portion of their shells, suggesting an aposematic function (Britson & Gutzke, 1993). On the other hand, at the intraspecific level, some freshwater turtles exhibit sexual dichromatism that is likely related to specific traits for partner recognition and quality assessment. For example, males of the North American freshwater turtle *Emydoidea blandingii* and the Asian turtle *Batagur borneoensis* possess chromatic details that may be used for both intraspecific and intersexual recognition (Moll *et al*. 1981; Rowe 1992). In addition to their social communication function, colors can give reptiles a thermal advantage by allowing faster heating rates (Clusella-Trullas 2008). For turtles, for example, a typically dark carapace would increase heat absorption during aerial thermoregulation (Boyer 1965; Rowe *et al*. 2014).

In this scenario, the question arises of how conspicuous coloration, which lacks chromatic distinction between sexes to the human eye, might influence the natural history of a turtle, such as *Ranacephala hogei*. This freshwater turtle occurs in South America, inhabiting rivers in the Atlantic Forest hotspot of southeastern Brazil (Assis *et al*. 2024). Its coloration includes a yellow plastron; dark red on the dorsal side of the females' heads; yellow on the beak, gular region, and ventral part of the neck; and pinkish-orange over a light cream background on the ventral region of the limbs and tail (Rhodin *et al*., 1982). Our observations of live specimens also reveal pinkish-orange tones on the plastron, dark gray on the dorsal side of the limbs and neck, and dark red on the dorsal side of the males' heads. To date, the coloration of *R. hogei* hatchlings remains unknown, and no studies have reported an objective analysis of the integument coloration of this species. However, turtles are well adapted to color vision, possessing the ability to perceive more shades or hues of long-wavelength light (Loew & Govardovskii 2001; Twyman *et al*. 2016), providing them with a different perception of color compared to humans. Therefore, the conspicuous coloration of *R. hogei* makes it an excellent model for investigating the influence of chromatic traits on its life history.

We emphasize that research in this area goes beyond understanding the natural history of *R. hogei*. For nearly three decades, this freshwater turtle has been present on red lists (Drummond *et al*. 2022; Vogt *et al*. 2023) and is currently one of the 25 most endangered turtle species in the world (TCC 2018). This status has made this species one of the few reptiles prioritized for maintenance and reproduction in captivity in Brazil, with the objective of maintaining a secure ex situ population (AZAB 2018). From this perspective, information about its coloration would help to understand its life history, aiding both in ex situ management and in conservation programs for wild populations. Therefore, we have characterized the coloration of *R. hogei* hatchlings and, using spectrophotometry techniques, have characterized the reflectance in the visible spectrum (400-750 nm) of the integument of adults and juveniles. Additionally, we have associated these results with observations of its natural history.

4.3. Materials and methods

Collection of turtles

We obtained specimens of *Ranacephala hogei* between 2016 and 2023 through a long-term field study (see methodology in Assis *et al*. 2024). The captures were made in the Paraíba do Sul River basins, and Itabapoana river, in the states of Rio de Janeiro, Espírito Santo and Minas Gerais, southeastern Brazil. We determined the sex and age (adult and juvenile) of the freshwater turtles based on the plastron concavity in males and the pre-cloacal tail length, which is longer in males compared to females (Rhodin *et al.* 1982). We also measured the carapace length using a caliper with a precision of 0.01 mm. Two specimens were used to characterize hatchling coloration, and nine specimens (three adult males, three adult females, and three juveniles) were used for spectral reflectance analyses (Table 1). Following the studies, we deposited one specimen of *R. hogei* in the Coleção de Répteis do Museu de Zoologia João Moojen (MZUFV) at the Universidade Federal de Viçosa, in the state of Minas Gerais, Brazil. The remaining specimens were released at the same capture site.

Table 1. Specimens of *Ranacephala hogei* used in the present study. Spec. = specimen. River basin: PS = Paraíba do Sul, ITB = Itabapoana. States: MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro. Sex: I = indeterminate, F = female, M = male. Life stage: H = Hatchling, J = juvenile, A = adult. Datum = WGS 84.

Spec.	Record date	River basin	Capturing site	Coordinates	State	Sex	Life stage	Carapace length (mm)	Research
1	2016	PS	Negro River	-21.7347 -41.9464	RJ	I	H	56.4	Hatchling color
2	2016	PS	Negro River	-21.7347 -41.9464	RJ	I	H	75.5	Hatchling color
3	2020	PS	Negro River	-21.7347 -41.9464	RJ	I	J	170.4	Spectral reflectance
4	2022	PS	Carangola River	-20.8519 -41.9999	MG	F	A	288.0	Spectral reflectance
5	2022	PS	Carangola River	-20.8519 -41.9999	MG	F	A	339.0	Spectral reflectance
6	2022	PS	Carangola River	-20.8519 -41.9999	MG	M	A	322.0	Spectral reflectance
7	2022	PS	Carangola River	-20.8519 -41.9999	MG	M	A	278.0	Spectral reflectance
8	2023	ITB	Itabapoana River	-21.1772 -41.5546	RJ/ES	I	J	211.1	Spectral reflectance
9	2023	PS	Carangola River	-20.8519 -41.9999	MG	M	A	327.5	Spectral reflectance
10	2023	PS	Carangola River	-20.8519 -41.9999	MG	I	J	132.4	Spectral reflectance
11	2023	PS	Negro River	-21.7347 -41.9464	RJ	F	A	303.0	Spectral reflectance

Spectral reflectance

We used an Ocean Optics USB2000 fiber optic spectrometer coupled with a 400 mm diameter UV-VIS reflection probe to measure spectral reflectance. The spectral reflectance of the turtle's tegument was measured between 400 and 750 nm at a fixed distance of 3 mm from the surface and at a 90° angle. To achieve this, we placed the tip of the reflection probe in a hollow sheath made of rigid rubber, with the flat tip touching the turtle's tegument. This setup ensured the elimination of ambient light, maintained a standardized distance between the probe and the skin, and provided a constant angle to minimize glare and specular reflection (Endler 1990). We calibrated the equipment using a white standard (Ocean Optics, WS-1-SS White Standard) and performed a dark current reading. The turtles were kept alive in clean water inside plastic buckets, and the surface of the tegument was dried with a cloth just before taking reflectance measurements. We manually restrained the freshwater turtles and measured spectral reflectance in nine regions of their integument, including the head, neck, plastron, limbs, and tail (Figure 1). For each specimen, we conducted five measurements on each of the selected regions and then calculated the average reflectance for each region.

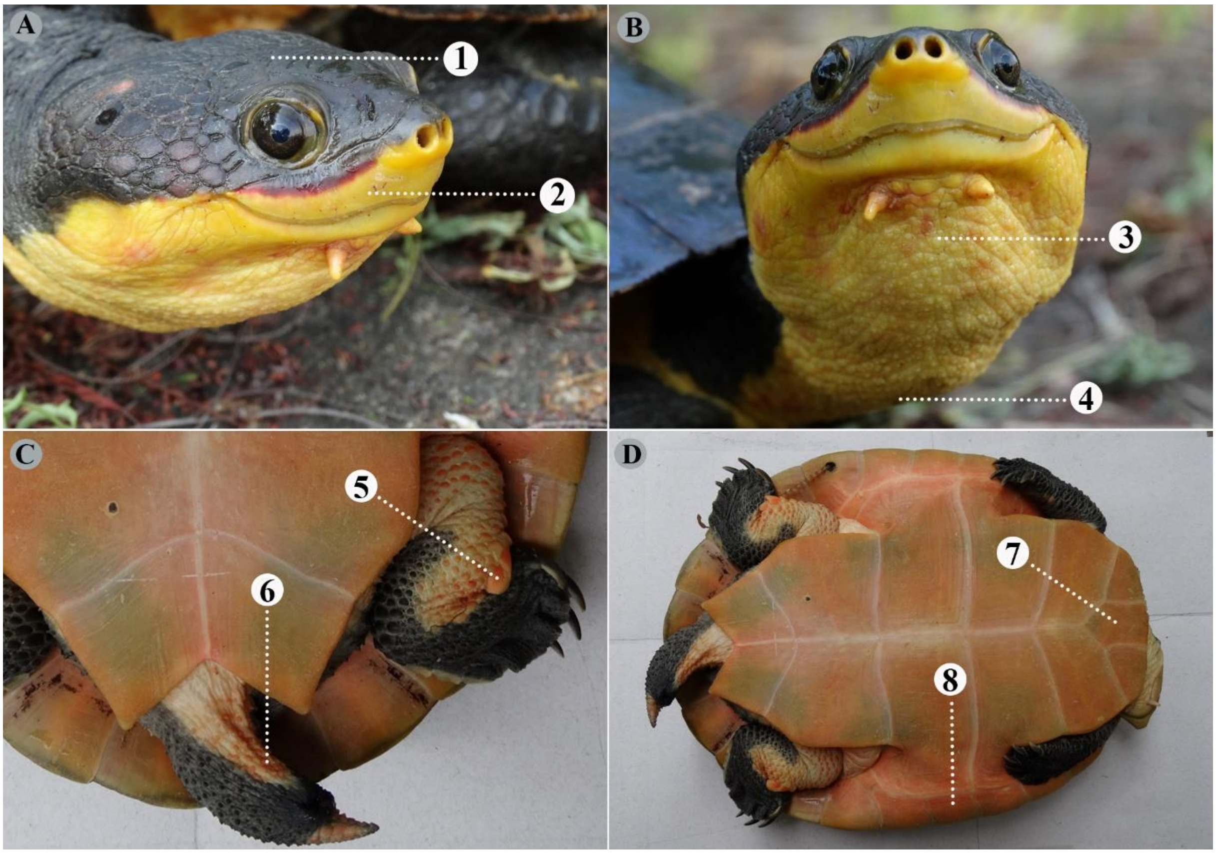

Figure 1. Views of two male *Ranacephala hogei* (A-B and C-D), highlighting the parts analyzed for spectral reflectance: A1 = dorsal head skin, A2 = upper beak, B3 = gular region, B4 = ventral surface of the neck, C5 = distal tibial scale, C6 = ventral surface of the tail, just after the cloaca, D7 = intergular shield, and D8 = ventral surface of the sixth left marginal shield.

Statistical analyses

We tested the potential intersexual and ontogenetic differences in color dominance and brightness by conducting individual factorial analysis of variance (ANOVA) for each measured body region. The assumptions of the model (normality and homoscedasticity) were met.

4.4. Results

Hatchling color

The hatchlings of *Ranacephala hogei* exhibit a dark dorsal coloration and a light ventral coloration, with the boundary between these colors starting at the middle of the nostrils, extending below the eyes, passing through the middle of the tympanums, and continuing along the side of the neck (Figure 2). The carapace is uniformly dark brown, while the plastron displays a strong pinkish-orange hue, with the ventral parts of the marginal scutes following the same color pattern. The larger hatchling already exhibited significant mineralization in the plastron, obscuring its bright colors. The dorsal and lateral part of the head is dark red, with a lighter red stripe extending below the eyes. The maxillae are yellow or light cream, and the gular region features small pinkish-orange spots on a light cream background. The iris is dark brown with a yellow border around the pupil. The dorsal part of the neck is grayish, whereas the ventral part mirrors the gular region's pattern. The ventral surface of the arm has a light cream background with pinkish-orange scales, while the hand and forearm are grayish. The ventral surfaces of the thigh and tibia exhibit a similar pattern to the forelimbs, with pinkish-orange scales on a light cream background. The dorsal parts of the legs and feet are grayish, and the dorsal part of the tail and cloaca are also grayish, with the base and tip of the ventral tail being pinkish-orange.

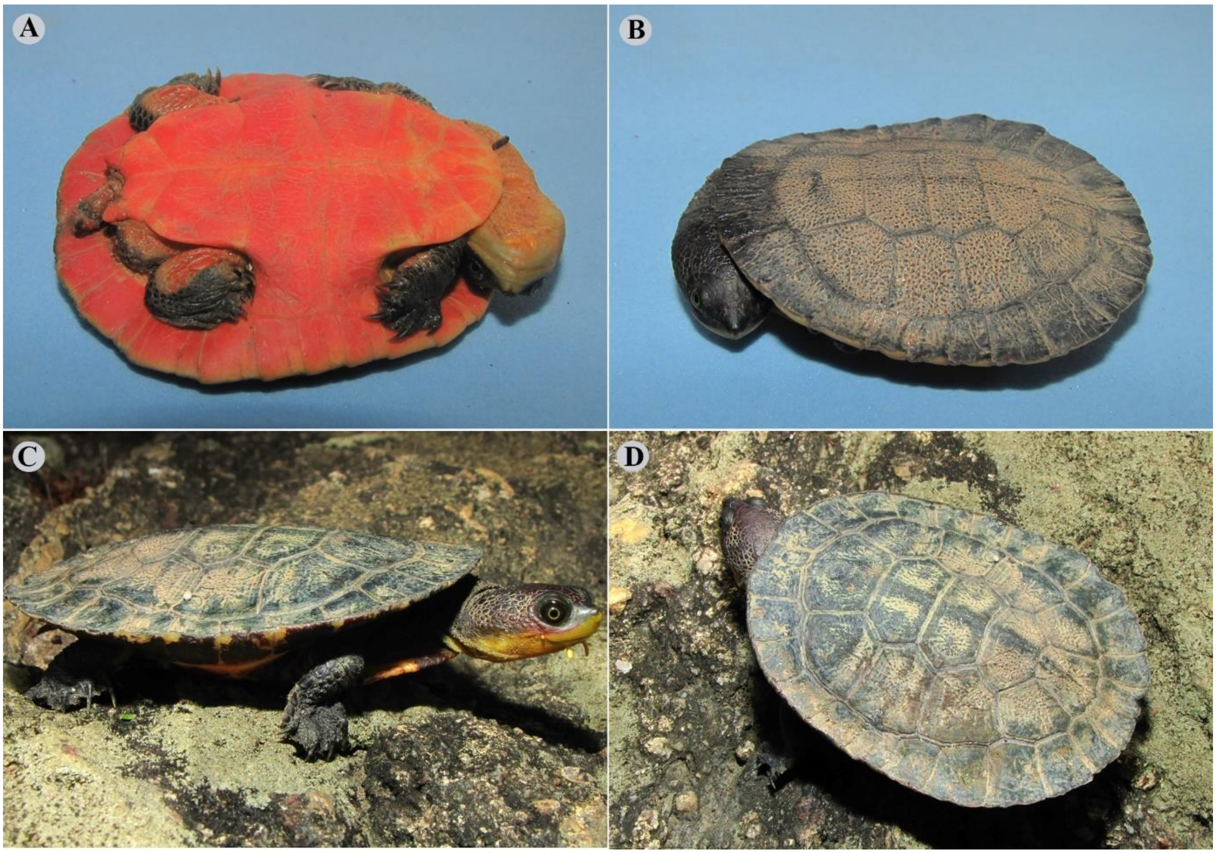

Figure 2. Hatchlings of *Ranacephala hogei*. A-B = ventral and dorsal view of specimen 1 (carapace length 56.4 mm), C-D = lateral and dorsal view of specimen 2 (carapace length 75.5 mm).

Spectral reflectance

The ANOVA detected no significant difference in color predominance among males, females, and juveniles. The dorsal surface of the head exhibited low reflectance with a peak in the 700-750 nm range, indicating a predominance of dark red coloration (Figure 3A). In contrast, the reflectance in other measured body areas ranged from medium to high, revealing lighter colors. Specifically, the upper beak, gular region, ventral neck area, and intergular shield showed reflectance peaks in the 580-600 nm range, indicating a predominance of yellow (Figures 3B to 3D, and 3G). On the tibial scale, the ventral surface of the tail, and the ventral surface of the sixth left marginal scute, reflectance peaks in the 600-650 nm range indicated a predominance of orange (Figure 3E to 3F, and 3H). Conversely, the ANOVA results revealed significant differences in color brightness among males, females, and juveniles across several body regions: the dorsal surface of the head ($F_{2,5211}$=7997, P=<0.001), the upper beak ($F_{2,5211}$=761, P=<0.001), the gular region ($F_{2,5211}$=54.2, P=<0.001), the ventral neck region ($F_{2,5211}$=292.5, P=<0.001), the tibial scale ($F_{2,5211}$=136.4, P=<0.001), the ventral surface of the tail ($F_{2,5211}$=656.1, P=<0.001), the intergular shield ($F_{2,5211}$=1101, P=<0.001), and the ventral surface of the sixth left marginal scute ($F_{2,5211}$=465.2, P=<0.001). In all measured regions, the reflectance spectra for males, females, and juveniles did not overlap at least in some parts of the spectra. Except for the sixth left marginal scute, females exhibited greater brightness than males, with particularly pronounced differences observed on the dorsal surface of the head, the ventral surface of the tail, and the intergular shield. Differently, juveniles were generally less bright than adults, with the sole exception being the dorsal surface of the head, which showed a particularly marked difference.

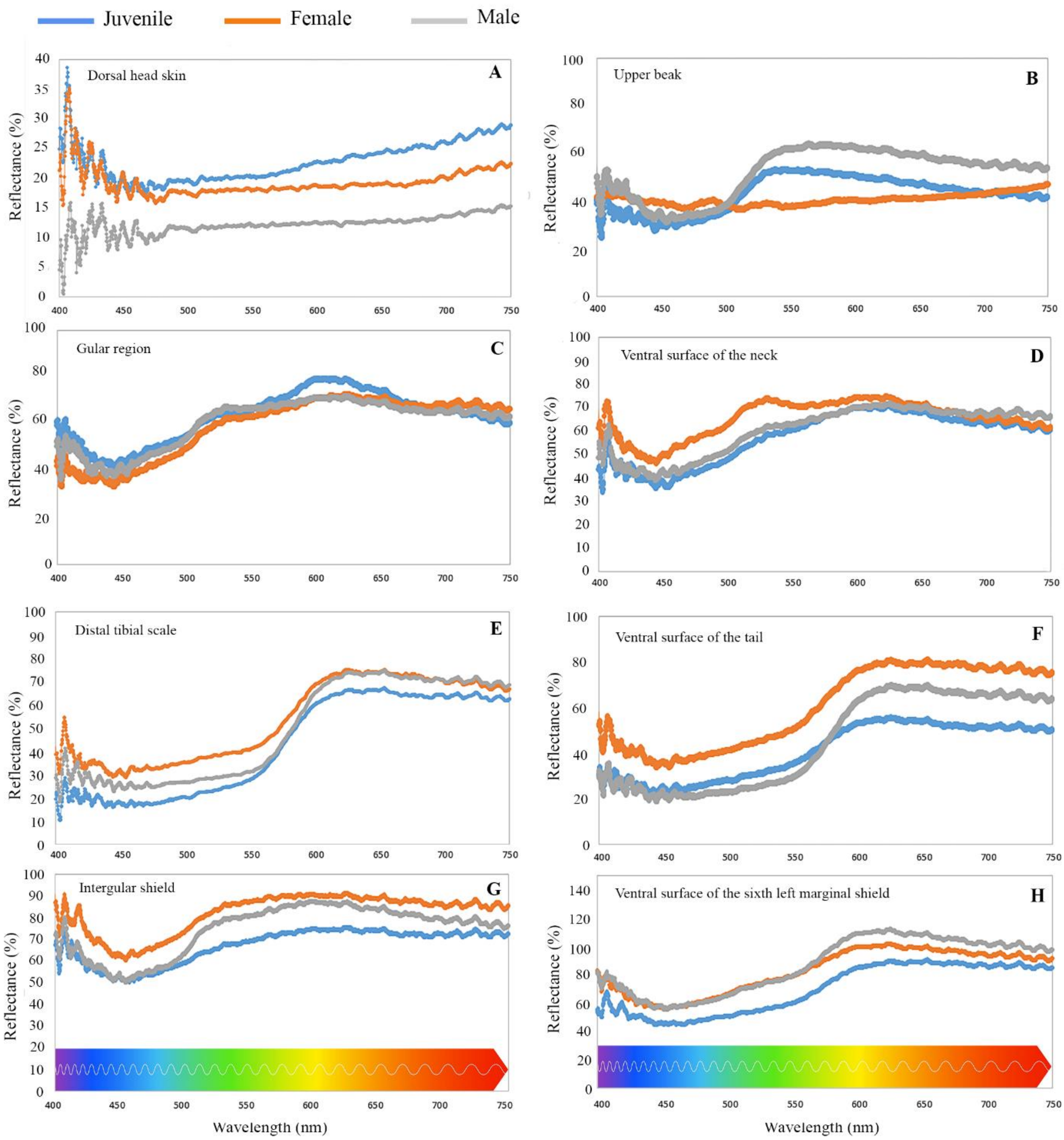

Figure 3. Spectral characteristics from different body parts of *Ranacephala hogei*.

4.5. Discussion

This study provides novel data on the conspicuous coloration of *R. hogei*, characterizing the color of hatchlings and, through reflectance measurements, precisely describing the magnitude of light reflected in juvenile, male, and female specimens. In the visible spectrum (400-750 nm), *R. hogei* did not exhibit sexual or ontogenetic dichromatism, showing differences only in the brightness (amount of reflected light) of the colors between these groups. Our results suggest that the conspicuous coloration of the species may influence its more discreet behavior and its preference for habitats with naturally turbid waters.

Overall, the coloration of *R. hogei* hatchlings is similar to that of adults, featuring a light-colored ventral surface and a dark dorsal surface. Differences from adults are observed in the ventral region, where hatchlings exhibit a brighter pinkish-orange coloration on the plastron, marginal scutes, hind limbs, and tail. Hatchlings of other freshwater turtle species also exhibit bright colors on their ventral surfaces, which generally become less vivid or dull as they mature into adults (Britson & Gutzke 1993; Ferronato & Molina 2009; Magnusson & Vogt 2014). These conspicuous colors may serve as aposematic signals indicating unpalatability, or function as Batesian mimicry by imitating unpalatable animals. However, despite the limited research on this topic in chelonians, conspicuous coloration in freshwater turtle hatchlings has not been demonstrated to function effectively as antipredatory signals in experiments with terrestrial predators and fish (Britson & Gutzke 1993; Reinke *et al.* 2017). An alternative explanation is that these bright colors may be caused by remnants of carotenoids present in the yolk, which are deposited on the ventral region in a manner that renders the visible color less detectable (Reinke *et al.* 2017). Notably, one of the hatchlings we examined exhibited strong mineralization of the plastron, with a predominant dark brown coloration. Indeed, if we consider this alternative explanation, the mineralization of the plastron in the *R. hogei* hatchling could be providing a survival advantage by concealing its conspicuous coloration.

The absence of chromatic differences, especially between the sexes in *R. hogei*, may indicate that their colors, despite being conspicuous, serve a camouflage purpose in the aquatic environment (Ross & Lovich, 1992). Its color pattern, with a dark dorsal region and a light ventral region, creates a counter-shading effect (Rowland *et al.*, 2008). Viewed from above, the dark carapace of *R. hogei* resembles the dark color of the substrate, and when viewed from below, its light-yellow plastron resembles the color of the sunlit sky. Additionally, light with different wavelengths is absorbed differently by water. Shorter wavelengths, such as blue and green, penetrate more deeply into the aquatic environment, while longer wavelengths, such as yellow and red, are absorbed within the first few meters of the surface (Smith & Baker 1979; Stomp *et al*. 2004). In this sense, the yellow, orange, and red colors present in *R. hogei* become dark or grayish in the first few meters below the water surface. This idea is reinforced by the reported habitat preferences of this freshwater turtle. *Ranacephala hogei* inhabits medium and low stretches of major rivers in plains where the altitude is low (average of 163 meters) and prefers backwaters near rapids (Vogt *et al*. 2023, Assis *et al*. 2024). Medium and low stretches of rivers in plains tend to have turbid waters due to the accumulation of sediments (Leopold *et al*. 1995), and this turbidity can be even greater in areas near waterfalls, as deposited sediments can be resuspended by the strong water flow (Allan & Castillo 2007). From this perspective, we can better understand the habitat preference of *R. hogei*. In turbid waters, the penetration of longer wavelength light is further reduced, making conspicuous coloration less visible and providing a survival advantage for the species. A similar phenomenon has been observed in the Asian freshwater turtle *Sacalia quadriocellata*, which reaches higher population densities in habitats where its better blends with the substrate (Xiao *et al*. 2016).

Despite the numerous examples of pronounced sexual dichromatism, subtle or imperceptible dichromatism to the human eye may play a functional role in communication and sexual selection (Ibáñez *et al*. 2013; Rossi *et al*. 2019). This appears to be the case for *R. hogei*, as our analyses revealed that females were brighter than males, particularly in the red coloration of the head, the orange of the ventral surface of the tail, and the yellow of the intergular shield. Although studies primarily highlight the role of males in sexual dichromatism, there is evidence that natural and sexual selection can directly influence the evolution of female coloration (Weiss *et al*. 2006; Ibáñez *et al*. 2013). For example, females of freshwater turtles *Mauremys leprosa* exhibit brighter coloration than males, and this brightness increases in healthier specimens (Ibáñez *et al*. 2013). Indeed, the red, orange, or yellow skin colors are determined by carotenoids from dietary sources (Alibardi 2013), allowing individuals to convey information about their health status (Polo-Cavia *et al*. 2012; Ibáñez *et al*. 2013, Ibáñez *et al*. 2014). In addition, males of *M. leprosa* are thought to be more exposed to predation due to increased activity during mate-searching, which may favor a duller coloration to avoid detection (Ibáñez *et al*. 2013). This raises the possibility that bright coloration in female *R. hogei* may serve a similar sexual function, particularly in an aquatic environment where light with different wavelengths is filtered. Since *R. hogei* inhabits naturally turbid waters (Vogt *et al*. 2023; Assis *et al*. 2024), increased brightness may enhance the chances of detection and recognition of females by males.

Juvenile *R. hogei* turtles, in contrast, exhibited less brightness than adults, particularly in the yellow region of the plastron and the orange areas of the limbs. This observation suggests that adults do not become more duller with age, as seen in other turtle species (Lovich *et al.* 1990) and supports the idea that the brighter coloration in female *R. hogei* may have a sexual function. Although our reflectance analysis did not include newly hatched hatchlings, it is possible that colors become more duller during the juvenile phase, with brightness increasing again in adulthood. This chromatic variation may reflect size-dependent predation pressure, as smaller turtles are more susceptible to predators than larger ones (Janzen *et al.* 2000). In this context, juvenile and hatchling *R. hogei* likely experience different predation pressures compared to adults, which could constrain the display of brighter colors. Consequently, reduced brightness might render smaller individuals less detectable to predators, while also enhancing light absorption during thermoregulation, benefiting their metabolism.

A curious finding was the low reflectance observed in the red part of the head of *R. hogei*, possibly an adaptation for thermoregulation. This freshwater turtle is considered rare (Mittermeier *et al.* 1980), and a recent extensive survey revealed that even in locations where it occurs, records of this species are difficult and scarce (Assis *et al.* 2024). In fact, during the long period of specimen collection for this study, we never observed *R. hogei* exposing itself, for example, to perform aerial thermoregulation. Our rare observations of the species in the wild were of animals floating just below the surface with their heads out of the water, which would dive to the bottom upon noticing our presence. Previous studies have suggested that more conspicuous individuals of a European freshwater turtle species (*Mauremys leprosa*) act more cautiously due to predator pressure (Ibanez *et al.* 2013, 2015). Thus, the interaction between conspicuous colors and discreet, evasive behavior may have led *R. hogei* to prefer aquatic thermoregulation. This preference would provide lower visibility and greater ease of escape, while exposing its head to the sun could contribute to its thermal regulation. The red coloration of its head showed an upward trend with a reflectance peak between 700-750 nm, close to the infrared. Longer wavelengths approaching the infrared spectrum tend to heat up more because this radiation is converted into thermal energy, in contrast to shorter wavelengths such as violet and blue (Modest 2013). The combination of this chromatic characteristic with low reflectance would increase heat absorption in the head of *R. hogei*, compensating for the reduced direct exposure of its body to the sun and decreasing the need for or duration of thermoregulation.

In conclusion, while this study is of descriptive nature, it establishes a foundation for new hypotheses aimed at understanding the relationships between selective pressures and the specific functions of coloration in *R. hogei*. In this context, we have demonstrated that the species' coloration exhibits a consistent pattern regardless of age or sex. However, we observed ontogenetic and sexual brightness dichromatism, with adults being brighter than juveniles and females being brighter than males, suggesting that female brightness may serve a sexual function. Additionally, we propose that, in an aquatic environment, the coloration of *R. hogei* may facilitate camouflage and thermoregulation, and that the species' chromatic pattern could be associated with its shy behavior and preference for turbid waters. From a conservation perspective, our data also provide a basis for some management recommendations for this threatened species. In an ex-situ context, it would be essential to provide a diet rich in carotenoids, as their coloration may be associated with sexual activity. Equally important would be to maintain turbid water in their enclosures or to add an aquatic plant cover over the water to provide a sense of security and minimize the animals' stress. Finally, in an in-situ context, conducting sampling in locations with higher water turbidity or during periods when river waters are more turbid may increase the chances of detecting *R. hogei*.

4.6. Acknowledgements

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5. CONCLUSÕES E RECOMENDAÇÕES

5.1. Distribuição

Após o longo período de estudos com o cágado-do-paraíba (*R. hogei*), constatamos que sua distribuição estava subestimada e que sua extensão de ocorrência abrange uma área significativamente maior do que se pensava. A maioria dos registros anteriores a este estudo foi realizada no rio Paraíba do Sul, principal curso d`água dessa bacia hidrográfica. No entanto, dentro dessa mesma bacia, *R. hogei* também ocupa rios de menor grandeza, como afluentes e subafluentes, alcançando regiões mais continentais. Dessa forma, é plausível que o mesmo padrão se repita em outras drenagens onde a espécie já foi registrada, como nas bacias dos rios Itabapoana e Itapemirim. Além disso, acrescentamos a bacia hidrográfica do rio Itabapoana como uma nova área de ocorrência confirmada para *R. hogei*. Acreditamos ainda que esse cágado possa estar presente em outras drenagens litorâneas, e recomendamos a realização de inventários ao norte da bacia do rio Itapemirim, e a sudoeste da bacia da Lagoa Feia.

5.2. Raridade de *Ranacephala hogei*

Ranacephala hogei é considerado um cágado raro nas regiões onde persiste, com distribuição altamente restrita. No entanto, observações de campo realizadas por nós e ainda não publicadas sugerem que *R. hogei* é uma espécie discreta e, mesmo em locais onde sua captura tem sido realizada, sua visualização é difícil. As raras observações feitas referem-se a indivíduos flutuando logo abaixo da superfície, que rapidamente mergulham para o fundo ao menor movimento do observador. Esse comportamento furtivo e evasivo pode levar *R. hogei* a passar despercebido, mesmo quando presente em determinada área, criando uma percepção artificial de raridade. Dessa forma, a espécie pode não ser tão rara quanto se presume. Essa aparente raridade pode ser um efeito de seu comportamento reservado, combinado à falta de amostragens de longo prazo e à aplicação limitada de métodos de detecção.

5.3. Persistência da espécie em suas áreas de ocorrência

No estado do Rio de Janeiro, há registros de *R. hogei* feitos nas décadas de 1950 e 1990, na calha do rio Paraíba do Sul, nos municípios de Pinheiral e Itaocara. No estado do Espírito Santo, seus últimos registros foram realizados em 1980, no rio Itapemirim. Já no estado de Minas Gerais, foi projetada, na década de 1990, a extinção de uma população de *R. hogei* no rio Carangola em menos de dez anos. No entanto, no presente estudo, registramos indivíduos jovens e adultos de ambos os sexos nessas localidades, indicando que a espécie ainda persiste e demonstra certa adaptabilidade às alterações ambientais. Esse padrão contrasta com o observado em outras espécies de quelônios mais sensíveis, que apresentam exigências mais especializadas de habitat e dieta. Ao revisitarmos essas áreas, constatamos uma paisagem tipicamente rural e dominada pelo homem, onde predominam pastagens e pequenas plantações, intercaladas por estreitas faixas de mata ciliar ou árvores esparsas ao longo dos rios.

5.4. A importância da ciência cidadã no estudo da espécie

Ao longo de sete anos de pesquisa, realizamos um extenso esforço de campo, com a instalação de armadilhas em 52 pontos distribuídos por quatro bacias hidrográficas, totalizando 125 dias/noites de amostragem. Além disso, visitamos seis coleções científicas, incluindo o Museu Nacional do Rio de Janeiro e o Museu de Zoologia da Universidade de São Paulo, duas das instituições mais relevantes do país. No entanto, por meio da ciência cidadã, obtivemos 21 novos registros de *R. hogei*, superando qualquer outro método empregado neste estudo. Esses registros revelaram sua presença em uma bacia hidrográfica adicional, ampliaram significativamente sua distribuição e evidenciaram sua persistência em áreas onde sua extinção havia sido prevista. Destacamos que, mesmo em regiões intensamente amostradas sem sucesso na captura, a ciência cidadã revelou ocorrências inéditas da espécie. Portanto, reforçamos a relevância da participação pública em estudos futuros sobre *R. hogei*, incluindo pescadores, comunidades ribeirinhas, agentes de fiscalização ambiental e observadores da natureza.

5.5. A função da coloração na camuflagem de *Ranacephala hogei*

A cores amarelas, vermelhas e alaranjadas, embora chamativas, desempenham importante papel na camuflagem de *R. hogei* no ambiente aquático. Sua região dorsal escura e a região ventral de coloração amarelo-clara criam um efeito de contrassombreamento. Isso faz com que o animal se confunda com o substrato escuro quando visto de cima, e se assemelhe ao céu iluminado quando observado de baixo. Além disso, concluímos que as cores conspícuas de *R. hogei* favorecem sua camuflagem subaquática, já que a água absorve de maneira diferenciada os comprimentos de onda da luz. Como os comprimentos de onda maiores, característicos do amarelo e vermelho, são absorvidos nos primeiros metros da coluna d’água, o animal assume coloração escura ou acinzentado logo nos primeiros metros abaixo da superfície. Esse fato pode justificar seu comportamento tímido e evasivo, evitando a exposição fora da água e fugindo para o fundo ao menor sinal de perigo. Além disso, essas conclusões podem explicar sua preferência por trechos médios e baixos de grandes rios em planícies de baixa altitude, especialmente em remansos situados abaixo de áreas encachoeiradas. Esses locais geralmente apresentam águas turvas em função do acúmulo de sedimentos, condição que se acentua pela ressuspensão causada pela força das corredeiras, favorecendo a sobrevivência da espécie.

5.6. Dicromatismo sexual e ontogenético de brilho

Nossas análises revelaram que as fêmeas de *R. hogei* são mais brilhantes que os machos. Esse resultado nos leva a acreditar que os machos estão mais expostos à predação em função do aumento da atividade durante a procura por parceiras, o que favorece a coloração mais opaca para evitar a detecção. Por outro lado, a coloração mais brilhante facilitaria a detecção e o reconhecimento das fêmeas pelos machos, especialmente em ambientes com águas turvas, onde a luz de diferentes comprimentos de onda é filtrada. Juvenis de *R. hogei*, por sua vez, exibiram brilho menor que os adultos, tanto machos quanto fêmeas. Embora nossa análise de reflectância não tenha incluído filhotes recém-eclodidos (aparentemente mais brilhantes), é possível que as cores se tornem mais opacas durante a fase juvenil, aumentando novamente o brilho na fase adulta. Essa variação cromática pode refletir pressões de predação dependentes do tamanho, já que tartarugas menores são mais suscetíveis a predadores do que as maiores. Além disso, cores opacas aumentam a absorção de luz durante a termorregulação, beneficiando seu metabolismo durante a principal fase de crescimento.

5.7. Coloração e termorregulação

Durante nossa longa amostragem de campo, nunca observamos *R. hogei* exposto para realizar termorregulação aérea. Nossas raras observações em vida livre foram de animais flutuando logo abaixo da superfície com a cabeça fora da água, que mergulhavam rapidamente ao perceber nossa presença. Acreditamos que a interação entre a coloração conspícua e o comportamento discreto e evasivo possa ter levado *R. hogei* a preferir a termorregulação aquática, e nossos resultados corroboram esse comportamento. As análises de reflectância mostraram que a coloração vermelha na cabeça da espécie, além de opaca, apresenta pico de reflectância próximo ao infravermelho. Sabemos que comprimentos de onda próximos ao infravermelho tendem a aquecer mais porque essa radiação é convertida em energia térmica. A combinação dessa característica cromática com baixa reflectância aumentaria a absorção de calor na cabeça de *R. hogei*, compensando a menor exposição direta do corpo ao sol e reduzindo a necessidade ou a duração da termorregulação. Assim, a termorregulação aquática proporcionaria menor visibilidade e maior facilidade de fuga, enquanto a exposição da cabeça ao sol poderia contribuir para uma absorção de calor mais eficiente.

5.7. A coloração no manejo ex situ e amostragens de campo

Do ponto de vista da conservação, nossos dados também fornecem uma base para algumas recomendações de manejo dessa espécie ameaçada. Em contexto ex situ, é essencial fornecer uma dieta rica em carotenóides, já que sua coloração pode estar associada à atividade sexual e possivelmente à saúde geral dos indivíduos. Igualmente importante é manter a água turva em seus recintos ou adicionar cobertura vegetal aquática à superfície da água, proporcionando uma sensação de segurança e minimizando o estresse dos animais. Essa estratégia também reduziria a visibilidade externa, evitando comportamentos de fuga constantes e melhorando o bem-estar dos exemplares em cativeiro. Além disso, a reprodução ex situ poderia ser favorecida por recintos ambientalmente complexos, que simulem características naturais de seu habitat, como correntes moderadas e áreas de remanso. Por fim, em contexto in situ, realizar amostragens em locais com maior turbidez da água ou em períodos em que os rios estejam mais turvos pode aumentar as chances de detecção de *R. hogei*. Essa informação é crucial para orientar futuras expedições de monitoramento e ampliar registros de ocorrência.