

Herpetofauna of the Estação Biologia Marinha Augusto Ruschi, a coastal forest remnant in the Atlantic Forest, south-eastern Brazil

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ABSTRACT – A four-day herpetofauna survey was undertaken monthly from October 2018 to September 2019 at the Estação Biologia Marinha Augusto Ruschi (EBMAR) nature reserve, a coastal forest remnant in south-eastern Brazil. Each survey involved active visual and auditory searching at night, pitfall traps and incidental observations. This study revealed 31 species of anurans and 28 reptiles. Swamp forest was the most used habitat by these species. Marginal vegetation was the most used microhabitat, followed by leaf litter and bromeliads. Regarding temporal occurrence, 27 (47%) species were occasional, 20 (34%) semi-constant and 11 (19%) constant. Three species are listed as Data Deficient (*Chelonoidis carbonarius*, *Physalaemus signifer* and *Sphaenorhynchus pauloalvini*) and two (3%) as Endangered (*Ameivula nativo* and *Arcovomer passarellii*). Thirty-seven (63%) species were recorded during the active survey whereas 11 (17%) were recorded in pitfall traps. The herpetofauna of EBMAR is spatially isolated because the surrounding landscape has been converted to agriculture and human settlement.

INTRODUCTION

Amphibians and reptiles are important components of biodiversity (Vitt & Caldwell, 2013), occupying different positions within the trophic chains (Teixeira & Coutinho, 2002; Alves et al., 2018; Zocca et al., 2017; 2019). The Brazilian Atlantic Forest biome is one of the world's biodiversity hotspots (Myers et al., 2000) with a high rate of endemism, harbouring about 720 species of anurans (Figueiredo et al., 2021) and 300 reptile species (Tozetti et al., 2017), with new species being described steadily (e.g. Mebert et al., 2020; Prates et al., 2020; Lacerda et al., 2021; Silva-Soares et al., 2021; de Sá et al., 2022; Neves et al., 2023; Zucchetti et al., 2023). Originally, the Atlantic Forest covered around 1,500,000 km², of which 76–89% were deforested (Ribeiro et al., 2009; Rezende et al., 2018). In recent decades, human-induced activities, mainly agriculture and urbanisation, have led to significant suppression and alteration of the Atlantic Forest (Tabarelli et al., 2010), causing local population extinction of amphibians and reptiles (Stuart et al., 2004; Becker et al., 2007; Böhm et al., 2013; Ferreira et al., 2016). Despite the high degree of urbanisation, coastal ecosystem remnants have rich herpetofauna, some of which have restricted distribution and are threatened with extinction (Tonini et al., 2011).

The Espírito Santo state, south-eastern Brazil is known to harbour 138 (22%) amphibian species (Rossa-Feres et al., 2017) and 134 (16%) reptile species (Guedes et al., 2023), but new species have been described in recent years (e.g.

Cardozo et al., 2018; Taucce et al., 2018; Maciel et al., 2019; Silva et al., 2020; Lacerda et al., 2021; Silva-Soares et al., 2021). Furthermore, many areas of Espírito Santo state have not been adequately sampled (Almeida et al., 2011) such as the coastal forest remnant of the Estação Biologia Marinha Augusto Ruschi. The surrounding landscape of this private reserve is mostly deforested due to the development of the real estate, agricultural and tourist activities, and proximity to large urban centres. Our objective was to elaborate a species list of herpetofauna from the Estação Biologia Marinha Augusto Ruschi and provide data on habitat and seasonal preferences.

MATERIALS & METHODS

Study area

The Estação Biologia Marinha Augusto Ruschi (hereafter EBMAR; 19° 58'09" S, 40° 08'37" W; Fig. 1) is a small 50 ha private reserve. It is located in the district of Santa Cruz, municipality of Aracruz, Espírito Santo state, south-eastern Brazil at altitudes ranging from 0 to 100 m. The vegetation cover of EBMAR is coastal Atlantic forest, and the climate is typical of a tropical savanna with a dry winter (Köppen classification Aw), with annual precipitation ranging between 1,300 to 1,600 mm and mean annual temperature of 22–24 °C (Xavier, 1998; Alvares et al., 2013).

We sampled three habitats (swamp forest, restinga forest and human-altered areas) and their respective microhabitats (water, shrub, bromeliad, human construction, leaf litter,

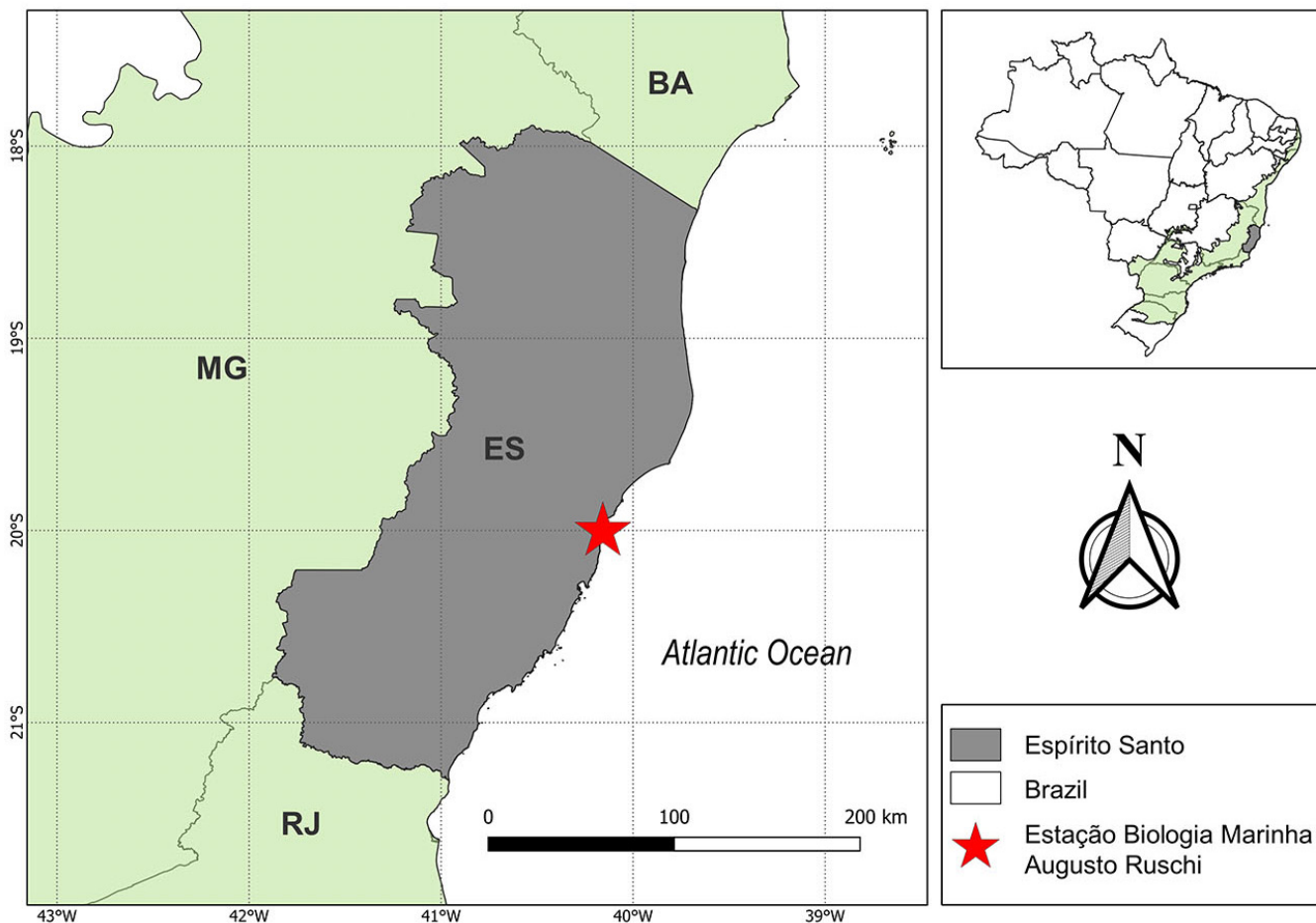


Figure 1. Location of the study site, Estação Biologia Marinha Augusto Ruschi (EBMAR), Santa Cruz district, municipality of Aracruz, south-eastern Brazil



Figure 2. Habitats and microhabitats sampled in the Estação Biologia Marinha Augusto Ruschi (EBMAR) - **A. & B.** Swamp forest, **C.** Restinga forest, **D.** Human-altered area

and marginal vegetation) (Fig. 2). The swamp forest is an ecosystem of the Atlantic Forest characterised by densely distributed vegetation, with tall trees and a large amount of decomposing plant material, forming a dense layer of leaf litter. Water availability in the swamp forest is greater than in other habitats, with temporary wetlands and periodically flooded areas. The restinga forest is an ecosystem of the

Atlantic Forest characterised by vegetation ranging from shrubs to large trees with a thin layer of leaf litter, and mostly arenaceous. Human-altered areas consist of human housing and horticulture with sparse vegetation, strips of bare sand, high solar incidence, and low potential for water retention.

Sampling

A four-day survey of the herpetofauna of EBMAR was undertaken at monthly intervals from October 2018 to September 2019. On each sampling occasion data were collected through active auditory and visual search, and pitfall traps. We sampled the herpetofauna by walking ~2 km transects at night (18:00 to 23:00 h) with three researchers, using active auditory and visual search method (Crump & Scott, 1994), resulting in a sampling effort of 240 h per researcher (720 h total). We also installed five stations of pitfall traps in the three habitats: swamp forest (2 stations), restinga forest (2 stations) and human-altered areas (1 station). Each station had seven 40-litre buckets arranged in a Y shape, connected by 5 m long and 1 m high black tarp drift fences (Cechin & Martins, 2000). We monitored the pitfall stations once a day during the sampling period, corresponding to a sampling effort of 1680 buckets (35 buckets/5 stations/4 days/12 months). We also included incidental observations of species by third-party and outside the study period.

Table 1. Number of individual anurans recorded from October 2018 to September 2019 in the Estação Biologia Marinha Augusto Ruschi (EBMAR), south-eastern Brazil. Number of individuals: brown shading = <15 individuals; green shading = 15–30 individuals; red shading = >30 individuals; Temporal occurrence: O= occasional; S= semi-constant; C= constant

Anuran species	Months												Temporal occurrence
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
Bufonidae													
<i>Rhinella crucifer</i>		■	■	■	■		■	■	■			■	S
<i>Rhinella granulosa</i>				■									O
Craugastoridae													
<i>Haddadus binotatus</i>	■	■	■	■	■		■	■	■	■	■	■	C
Hylidae													
<i>Boana albomarginata</i>		■	■	■			■	■	■	■	■	■	S
<i>Boana faber</i>		■	■	■	■	■	■	■	■	■	■	■	C
<i>Boana pombali</i>		■	■	■	■			■	■		■	■	S
<i>Boana semilineata</i>		■										■	O
<i>Dendropsophus berthalutzae</i>		■							■				O
<i>Dendropsophus bipunctatus</i>					■								O
<i>Dendropsophus branneri</i>	■	■	■	■	■		■	■	■	■	■	■	C
<i>Dendropsophus elegans</i>		■	■	■	■		■	■	■	■	■	■	S
<i>Dendropsophus sp. (cf. haddadi)</i>		■	■	■	■	■	■	■	■	■	■	■	C
<i>Itapotihyla langsdorffii</i>	■	■	■	■	■	■	■	■	■	■	■	■	C
<i>Nyctimantis brunoi</i>	■	■	■	■	■		■	■	■	■	■	■	C
<i>Ololygon argyreornata</i>		■	■	■	■		■	■	■	■	■	■	S
<i>Phyllodytes luteolus</i>	■	■	■	■	■	■	■	■	■	■	■	■	C
<i>Phyllomedusa burmeisteri</i>				■									O
<i>Scinax alter</i>	■	■	■	■	■	■	■	■	■	■	■	■	C
<i>Scinax cuspidatus</i>				■		■					■		S
<i>Scinax fuscovarius</i>		■											O
<i>Sphaenorhynchus pauloalvini</i>		■											O
<i>Sphaenorhynchus planicola</i>		■											O
<i>Trachycephalus nigromaculatus</i>				■									O
Leptodactylidae													
<i>Leptodactylus fuscus</i>		■											O
<i>Leptodactylus latrans</i>	■	■	■	■	■	■	■	■	■	■	■	■	C
<i>Leptodactylus natalensis</i>				■	■	■	■	■	■	■	■	■	S
<i>Physalaemus signifer</i>		■	■	■	■	■	■	■	■	■	■	■	C
Microhylidae													
<i>Arcovomer passarellii</i>		■						■	■				O
<i>Chiasmocleis capixaba</i>	■	■	■	■			■	■	■				S
<i>Stereocyclops incrassatus</i>		■											O
Odontophrynidae													
<i>Proceratophrys laticeps</i>	■	■	■		■				■		■		S

We classified the spatial distribution of the species according to habitat (swamp forest, restinga forest and human-altered areas) and microhabitat (water, shrubs, bromeliad, human housing, leaf litter and marginal vegetation). We classified temporal occurrence of species recorded during the sampling period as:

- i) occasional (occurring from one or two months);
- ii) semi-constant (occurring from three to nine months);
- iii) constant (occurring from 10 to 12 months) (Prado & Pombal, 2005).

The conservation status of the species follows the international (IUCN, 2023), national (ICMBIO/MMA, 2018)

and Espírito Santo (ES) state lists (Bérnils et al., 2019; Ferreira et al., 2019). We identified the species using photographic guides (Haddad et al., 2013) and comparison of specimens deposited in the Coleção de Zoologia do Museu de Biologia Prof. Mello Leitão (MBML). The voucher specimens were euthanised with lidocaine, fixed in 10% formalin, conserved in 70% alcohol (McDiarmid et al., 1994) and deposited in the MBML from the Instituto Nacional da Mata Atlântica (INMA) located in the municipality of Santa Teresa, Espírito Santo, Brazil. A list of these voucher specimens is provided in the Supplementary Material.

Table 2. Number of individual reptiles recorded from October 2018 to September 2019 in the Estação Biologia Marinha Augusto Ruschi (EBMAR), south-eastern Brazil, legends as per Table 1

Reptile species	Months												Temporal occurrence	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep		
Testudinidae														
<i>Chelonoidis carbonarius</i>														O
Amphisbaenidae														
<i>Leposternon</i> sp.														O
Dactyloidae														
<i>Dactyloa punctata</i>														S
<i>Norops fuscoauratus</i>														O
<i>Norops ortonii</i>														O
Gekkonidae														
<i>Hemidactylus mabouia</i>														C
Gymnophthalmidae														
<i>Leposoma scincoides</i>														S
Mabuyidae														
<i>Brasiliscincus agilis</i>														O
<i>Psychosaura macrorhyncha</i>														O
Phyllodactylidae														
<i>Gymnodactylus darwinii</i>														S
Polychrotidae														
<i>Polychrus marmoratus</i>														S
Teiidae														
<i>Ameiva ameiva</i>														S
<i>Ameivula nativo</i>														O
<i>Salvator merianae</i>														S
Tropiduridae														
<i>Tropidurus torquatus</i>														S
Boidae														
<i>Boa constrictor</i>														S
<i>Corallus hortulana</i>														S
Colubridae														
<i>Chironius foveatus</i>														O
<i>Drymoluber dichrous</i>														O
<i>Leptophis ahaetulla</i>														S
<i>Oxybelis aeneus</i>														O
<i>Spilotes sulphureus</i>														O
Dipsadidae														
<i>Dipsas indica</i>														O
<i>Dipsas newwiedii</i>														O
<i>Oxyrhopus petolarius</i>														O
<i>Philodryas olfersii</i>														O
Elapidae														
<i>Micrurus corallinus</i>														O
Viperidae														
<i>Bothrops jararaca</i>														S

RESULTS

We recorded 3785 individuals of the herpetofauna comprising 31 species of anurans (17 genera and seven families) and 28 species of reptiles (26 genera and 15 families) at EBMAR; the species names and families are shown in Tables 1 and

2. Photographs of many of these species are shown in the Supplementary Material - Table 1S for anurans and Table 2S for reptiles. The details of the species, the habitats and microhabitats in which they were encountered, their endemism and the sampling methods that revealed their presence are shown in Supplementary Material - for anurans

(Table 1S) and for reptiles (Table 2S). The most recorded anuran species were *Phyllodytes luteolus* (674 records; 19%), *Physalaemus signifer* (612 records; 18%) and *Scinax alter* (473 records; 14%). The most recorded reptile species were the lizards *Tropidurus torquatus* (102 records; 31%) and *Ameiva ameiva* (94 records; 29%).

Regarding habitat, 45 (76%) species were recorded in the swamp forest, 38 (64%) species in human-altered areas and 18 (30%) species in the restinga forest. The distribution and overlap of species between habitat types is shown in Figure 3. Twelve amphibian species were found in all three habitat types compared with only three reptile species. No amphibian and only one reptile species was restricted to the restinga.

Regarding microhabitat, 1364 (36%) individuals were recorded in the marginal vegetation of the water bodies, 1187 (31%) individuals were in the leaf litter, 785 (21%) individuals were in bromeliads, 234 (6%) individuals were in shrubs, 168 (4%) individuals were in the water, and only 47 (1%) individuals were recorded in human constructions. Thirty-seven (63%) species were recorded in only one microhabitat, 12 (20%) species were in two microhabitats, and eight (14%) species were in three microhabitats (Tables 1S and 2S). The highest number of species was recorded in the leaf litter (N = 28 species; 47%), followed by shrub (N = 20 species; 34%) and marginal vegetation (N = 18 species; 30%).

Thirty-seven (63%) species were recorded during active survey (visual and auditory), followed by 11 (19%) species recorded through pitfall trap. Six (10%) species of anurans and 15 (25%) species of reptiles were incidental records (Tables 1S and 2S). Active search (i.e. auditory and visual) recorded the highest number of individuals (N = 3629 individuals; 96%), followed by pitfall trap (N = 98 individuals; 3%), and incidental sightings (N = 58 individuals; 1%).

Regarding temporal occurrence, 28 (47%) species were occasional, 20 (34%) semi-constant and 11 (19%) constant (Tables 1 and 2). Overall, constant species had more individuals (>30 individuals) per month. *Itapotihyla langsdorffii*, *Nyctimantis brunoi*, *P. luteolus* and *S. alter* were active every month of the year. The lowest number of species (N = 13; 22%) was recorded in March, whereas the highest number of species (N = 31; 52%) was recorded in November (Tables 1 and 2).

We recorded 32 species in EBMAR (54% of the total) that are endemic to the Atlantic Forest, of which 24 were anurans (41%) and eight reptiles (14%) (Tables 1S and 2S). Regarding conservation status, 51 (86%) species were categorised as Least Concern, three (5%) as Data Deficient (*Chelonoidis carbonarius*, *P. signifer*, *Sphaenorhynchus pauloalvini*), and two (3%) as Endangered (*Ameivula nativo*, *Arcovomer passarellii*) from either IUCN, Brazilian or state redlists.

DISCUSSION

Our survey of EBMAR provided a list of 31 anuran species and 28 reptile species. The 31 anuran species corresponds to 86% of the 36 species listed for the municipality of Aracruz (Almeida et al., 2011), 23% of the 138 species listed for Espírito Santo state (Rossa-Feres et al., 2017), 4% of the 720 species listed for Atlantic Forest (Figueiredo et al., 2021),

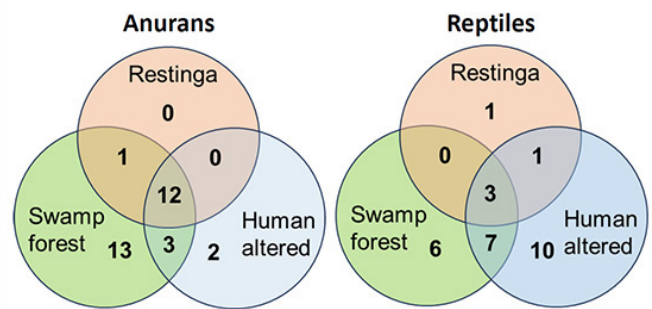


Figure 3. Number and overlap of herpetofauna species found in the three habitats at the Estação Biologia Marinha Augusto Ruschi (EBMAR)

and 3% of the 1188 species listed for Brazil (Segalla et al., 2021). The 28 reptile species corresponds to 21% of the 134 species listed for Espírito Santo state (Guedes et al., 2023), 9% of the 300 species listed for Atlantic Forest (Tozetti et al., 2017), and 3% of the 856 species listed for Brazil (Guedes et al., 2023). Studies at other sites in the Espírito Santo state have recorded both fewer species than EBMAR, such as Canal de Itaputanga (ca. 115 km south; N = 9 species; Maioli et al., 2018), Universidade Federal do Espírito Santo (ca. 38 km south; N = 20 species; Ferreira & Mendes, 2010), and more species than EBMAR, such as Reserva Biológica de Duas Bocas (ca. 50 km south; N = 76 species; Tonini et al., 2010) and Reserva Natural Vale (ca. 90 km north; N = 120 species; Bérnils et al., 2014; Almeida & Gasparini, 2015). However, most studies in Espírito Santo state were undertaken in large forest remnants and in protected areas. In contrast, EBMAR is a private, small and isolated forest remnant. The species richness of herpetofauna from EBMAR may be related to the high habitat heterogeneity, which includes permanent and temporary water bodies, an abundance of tank bromeliads, and forest with dense leaf litter.

Regarding habitat, the highest number of species (76%) were recorded in the swamp forest which is particularly rich in hylid species that use the permanent and temporary water bodies in this forest for breeding (Haddad et al., 2013; Silva et al., 2019). Surprisingly, human-altered areas were used by 38 (64%) of amphibian and reptile species of which 12 (20%) were found only in this habitat. Human-altered areas are found within the natural areas of EBMAR, thus species can move easily from natural to altered habitats. Furthermore, some species recorded in this habitat are considered to have high ecological plasticity, such as *Rhinella granulosa* and *T. torquatus*.

Regarding microhabitats, the marginal vegetation along the water bodies had the highest number of individuals. The high abundance of hylids was mostly associated with permanent and temporary water bodies surrounded by marginal vegetation in the swamp forest. Species of this family are characterised by adhesive discs present on the fingertips, which give them the ability to perch on vertical marginal vegetation (Cardoso et al., 1989; Zocca et al., 2014). Nine species (*Rhinella crucifer*, *Boana pombali*, *I. langsdorffii*, *N. brunoi*, *P. luteolus*, *S. alter*, *Ololygon argyreornata*, *Bothrops jararaca* and *Hemidactylus mabouia*) were recorded in bromeliads, showing the importance of this microhabitat for

the local species. *Phyllodytes luteolus* was the most recorded species in bromeliads, because it breeds in the accumulated rainwater between the leaves of these plants.

The use of multiple sampling methods may have increased the number of species recorded. Active survey recorded a higher number of individuals than either pitfall traps or incidental records. Active survey possibly detected more species because searching directly in refuges, burrows, bromeliads may reveal both active and inactive species (Auricchio & Salomão, 2002). Pitfall traps were responsible for recording species associated with leaf litter that generally have fossorial habits and are rarely observed during the active search or by incidental observation.

The EBMAR is a small forest fragment (ca. 4 km²), surrounded by plantations and human constructions. Despite this, EBMAR harbours higher species richness than other larger areas in the state of Espírito Santo, including threatened species such as *A. passarellii* (EN) and *A. nativo* (EN) (MMA, 2014; Bérnils et al., 2019). *Arcovomer passarellii* was abundant and widely distributed across the sampled habitats. On the other hand, only one individual of *A. nativo* was recorded, which was found in the restinga forest. Both species had preference for the leaf litter microhabitat, highlighting the importance of forest remnants to maintain these populations. It is noteworthy that records of the hylid *B. pombali*, the tortoise *C. carbonarius* and two snakes *Boa constrictor* and *Corallus hortulana* were the first for the municipality of Aracruz. Although a native of the Atlantic Forest, *Chelonoidis carbonarius* has become locally extinct in most of its range. It is likely *C. carbonarius* has been released in EBMAR by the local animal rehabilitation centre (CEREIAS).

Our study has shown that EBMAR supports a rich herpetofauna, highlighting the conservation importance of this isolated remnant of coastal Atlantic Forest, surrounded by a low permeable matrix (i.e. human settlements and agriculture). The understanding of the composition of the EBMAR herpetofauna fills knowledge gaps about habitat use, microhabitat selection and temporal occurrence. The long-term monitoring of these species should be undertaken as a basis for the elaboration of effective conservation plans.

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