Experimental introduction of *Liolaemus lutzae* (Squamata: Iguanidae) in Praia das Neves, State of Espírito Santo, Brazil: a descriptive study 18 years later

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ABSTRACT. This article examines the results of the introduction into Praia das Neves, state of Espírito Santo, Brazil, of *Liolaemus lutzae* Mertens, 1938, a lizard species threatened with extinction. Since there are few studies that evaluate how species establish and adapt to new environments, it is useful to assess to what extent the introduction of a critically endangered species into an area similar to where it originally occurred can help reduce its decline in number of individuals and avoid its possible extinction. This study presents the first results of an ongoing monitoring survey set up after the experimental introduction. We analyze how the introduced population uses space and food and we compare these characteristics to that of the original population at Barra de Maricá, Rio de Janeiro state. We also compare morphological measurementss of specimens from both populations. Both make similar use of the microhabitat, but there are differences in their diets. We recommend that the introduced population, potential competitors, predators, parasites, and the habitat characteristics continue to be monitored, so as to insure that this species will not become a threat to Praia das Neves beach community.
KEY WORDS. Atlantic Forest; conservation; habitat-specialist; lizard; restinga; sand-dune.

RESUMO. **Introdução experimental de *Liolaemus lutzae* (Squamata: Iguanidae) em Praia das Neves, Espírito Santo, Brasil: um estudo descritivo 18 anos depois.** Esse artigo examina o resultado da introdução, em Praia das Neves, Estado do Espírito Santo, Brasil, de *Liolaemus lutzae* Mertens, 1938, uma espécie de lagarto ameaçada de extinção. Como existem poucos estudos que avaliam como espécies estabelecem-se e adaptam-se a novos ambientes, é útil conhecer em que extensão a introdução de uma espécie criticamente em perigo em uma área similar àquela onde ocorria naturalmente pode ajudar a reduzir seu declínio em número de indivíduos e evitar sua possível extinção. Esse estudo apresenta os primeiros resultados de um monitoramento em andamento, iniciado depois da introdução experimental. Analisamos o uso do espaço pela população introduzida e sua alimentação, e comparamos essas características com a da população original em Barra de Maricá, Estado do Rio de Janeiro. Também comparamos as medidas morfológicas das duas populações. Ambas fazem uso similar do microhabitat, mas há diferenças nas suas dietas. Recomendamos que a população introduzida e seus competidores potenciais, predadores e parasitos, e as características do habitat continuem a ser monitorados, para garantir que essa espécie não se torne uma ameaça à comunidade de Praia das Neves
PALAVRAS-CHAVE. Conservação; dunas; Floresta Atlântica; habitat-especialista; lagarto; restinga.

The commercial exploitation of the Atlantic Forest biome in Brazil began with the arrival of the Europeans, who brought with them new plant species, cattle, and other exotic domestic animals. However, in the last thirty years, the destruction has been so intense as to lead to major shifts in the forest ecosystem. Today, only 8% of the original 1.350.000 km^2 of forest cover remain (Fundação SOS Mata Atlântica & Inpe 2002). The biological wealth of this biome, coupled with the threat that hangs over it, make it a conservation priority (Myers *et al*. 2000a). The natural insularity of the open vegetation that covers the sand dunes along the coast explains the uniqueness of the area's endemic lizard fauna. Yet, these *restinga* areas have been devastated by urban occupation and preserved restinga vegetation is now restricted to small and isolated fragments. To this day, few restinga areas are under Federal or State protection and, therefore, their flora and fauna are in urgent need of a full scale conservation plan.

One of their threatened species is *Liolaemus lutzae* Mertens, 1938, a small, white sand lizard included in the Brazilian list of critically endangered species (Ibama 2003) and in the IUCN red list as vulnerable (Iucn 1994). *Liolaemus lutzae* is endemic to the *restingas* of Rio de Janeiro, and can be found

from the Restinga da Marambaia (23º05'S, 44º00"S – southern limit) to Cabo Frio (22º53"S, 42º00"W – northern limit).

Liolaemus lutzae prefers sand dunes closer to the sea, but can also be found at the edge of scrub habitats (Araujo 1984, Rocha 1991). Since this lizard is very dependent on beach habitats, its distribution has probably been historically determined by sea level fluctuations in the Quaternary (Vanzolini & Ab'Saber 1968). The rise of sea level due to global climate change (Mitrovica *et al*. 2006) is likely to affect current populations of this sand lizard species.

Rocha & Bergallo (1992) studied the population of *L. lutzae* in Barra da Tijuca, within the urban area of the Rio de Janeiro municipality, in 1982 and 1991. They monitored the decrease in population size during that period and suggested that this decline was due to decreased availability of the vegetation used by the species, as a result of human interference. The population of *L. lutzae* in Barra da Tijuca became restricted to a four kilometer stretch of beach in the Biological Reserve of Barra da Tijuca (Rocha & Bergallo 1992). Road construction in the *restinga* and urban expansion have altered the beach landscape and contributed to large-scale population fragmentation throughout the species range.

The natural fragmentation of the beaches, due to the large mountain ranges that extend to the sea, suggests restricted natural gene flow among white-sand lizard populations. This special condition increases the risk of extinction. To study their colonization ability, in 1986, A.F.B. Araujo and others transferred, on an experimental basis, fifty one *L. lutzae* individuals from the beach at Barra de Maricá (Rio de Janeiro state) to the beach of Praia das Neves, state of Espírito Santo (Fig. 1). The lizards were collected by hand, and measurements were made of snout-vent length and head width. Twenty males, twenty females and eleven juveniles were taken to Praia das Neves in plastic recipients. All lizards were released at the same time, in a single site. Translocation of the lizards from Barra de Maricá to Praia das Neves took five days. Niche relationships between the eight lizard resident species of Praia das Neves *restinga* habitats and their common predators were known (Costa *et al*. 1990) and the researchers supposed that an open niche was available for this white sand lizard in the beach habitat at Praia das Neves.

Despite the conservation precautions taken by the researchers, the experiment is open to criticism because it was not preceded by a demographic study covering all potential interacting populations. Nevertheless, the transferred population remained in its new locality of distribution, at first sight using the space as it is used in the original site. Researchers have been studying the lizard communities in the two areas and were aware of the similarities between Praia das Neves and Barra de Maricá. Today, the Brazilian Government sponsors the "Management of the white-sand-dune lizard, *Liolaemus lutzae* project" ("Manejo da lagartixa-de-areia, *Liolaemus lutzae*"), designed to protect *L. lutzae*. This conservation program led by MMA/FNMA is being developed in collaboration with the Universidade Federal Rural do Rio de Janeiro (UFRRJ/FAPUR) (IBAMA/SISBIO n.13980-1).

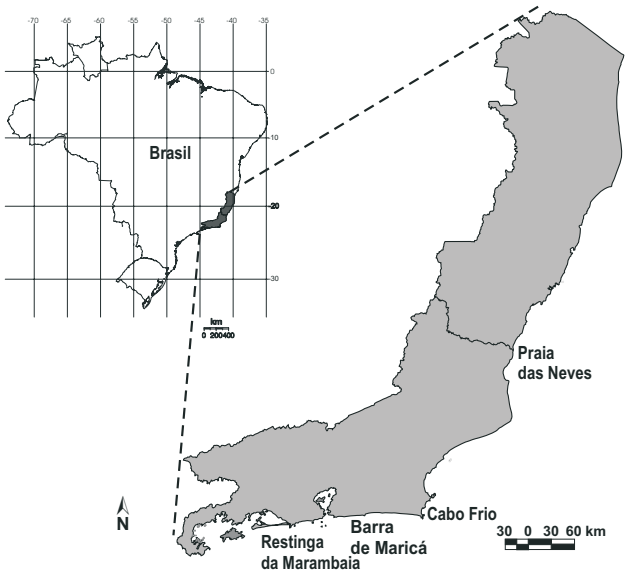

Figure 1. Geographic localization of the two studied populations of *Liolaemus lutzae* in Barra de Maricá and Praia das Neves. Restinga da Marambaia and Cabo Frio are the species distribution extremes.

There are few publications on the ability of threatened species to establish themselves and adapt to new environments but this procedure has been used as a conservation strategy (Knapp 2001, Sullivan *et al*. 2004). Although this paper does not deal specifically with the threat to the existing community, it can contribute to a better understanding of the adjustment process of the threatened species. We believe a long term monitoring program of the status of the introduced *L. lutzae* can assist us in formulating alternative conservation strategies to avoid the white-sand-dune lizard decline and possible extinction.

In this paper we present the first monitoring results of this experimental introduction and discuss how the introduced population uses space and food. We also compare these ecological attributes to those of the Barra de Maricá founder population. Finally, we examine morphological differentiation between individuals from both populations.

MATERIAL AND METHODS

We monitored the introduced population in Praia das Neves beach, in Presidente Kennedy municipality (22º57'49, 39"S, 42º51'11,6"W), during three field trips (fifteen days each) in September 2002, December 2002 and February 2003. We compared morphological data collected in Praia das Neves with morphometrics from the 51 lizards introduced in 1986. We also analyzed the diet of the introduced population and compared with the diet data published by Rocha (1989).

We searched for lizards in the beach habitat and in neighboring *restinga* scrubs. One hundred and thirty seven lizards were captured by hand, measured (caliper 0.1 mm) and weighed (dynamometer 0.5 g). In order to establish comparisons with the 1986 Barra de Maricá population, we measured snout-vent length (SVL) and head width (HW). We also recorded the sex, the microhabitat used by each lizard, and their geographical position (GPS Garmin 12 XL). We used the following microhabitat categories: open sand, sand hole, plastic, Styrofoam, rubber, paper, pet/plastic bottle, wood and vegetation. All lizards were marked by toe-clipping and released. To test if space niche is conservative amongst *L. lutzae* populations, in May 2003 we obtained data on habitat use in Barra de Maricá, following the same protocol described above, from the same site where the introduced population was captured.

To obtain comparable reproductive data from the introduced white-sand-dune lizard, thirty lizards from Praia das Neves beach were killed with a lethal dose of Tyopenthal (permit 009/03 RAN – IBAMA, process 02010.001019/03-31). They were fixed in formalin 10%, stored in ethanol 70%, and later dissected and their reproduction condition assessed. Females presenting vitellogenic follicles and *corpora lutae* (reproductive non-ovigerous) or at least one oviductal egg (ovigerous) were considered reproductive. Males with convoluted epididymis were considered reproductive. We also analyzed stomach contents in the laboratory. The frequency of food items in the Praia das Neves beach diet was compared to the Barra de Maricá diet data published by Rocha (1989).

To evaluate the impact of the eighteen years in the new environment on body proportions, we conducted a covariance analysis, comparing the head length (HL) of lizards from Barra de Maricá and Praia das Neves, using SVL as covariate. Differences in prey volume between sites and in the use of microhabitat categories between Praia das Neves beach and Barra de Maricá were tested using the Kolmogorov-Smirnov Test. We tested the normalization of the data, and all analyses were conducted using Systat 7.0, level of significance of 5% (Zar 1999).

RESULTS

The introduced population successfully colonized the Praia das Neves beach. During the monitoring effort, we captured 137 individuals and recaptured two. The population was grouped in three distinct sites along the 15 km of the beach (sites 1, 2 and 3, Fig. 2). One hundred and thirty five lizards were registered between sites 1 and 2 (Fig. 2), while only two were seen at site 3, about 14 km from the introduction site ("O"). In all registers, *L. lutzae* occupies the same habitat as the species in the *restingas* of Rio de Janeiro. No *L. lutzae* were recorded out of the beach.

The covariance analysis shows that the two populations (Praia das Neves beach and Barra de Maricá) do not differ in body proportions (Tab. I) ($F = 1.734$, $p = 0.190$), although the regression coefficient "r" was significant (SVL as covariate, $r = 0.956$, $F = 1880.224$, $p < 0.001$). Nevertheless, the composition of the *L. lutzae* diet (volume of items) is not similar between these two populations (Kolmogorov-Smirnov, D max = 0.3361, $p < 0.01$). Both populations present a varied diet, but in Maricá they consume more prey types. Plant material was the most important item in lizard diet for both areas (Tab. II).

The two populations do not differ in the use of microhabitats (Kolmogorov-Smirnov, D max = 0.500, $p = 0.188$) (Fig. 3). We found four other lizard species in the beach habitat,

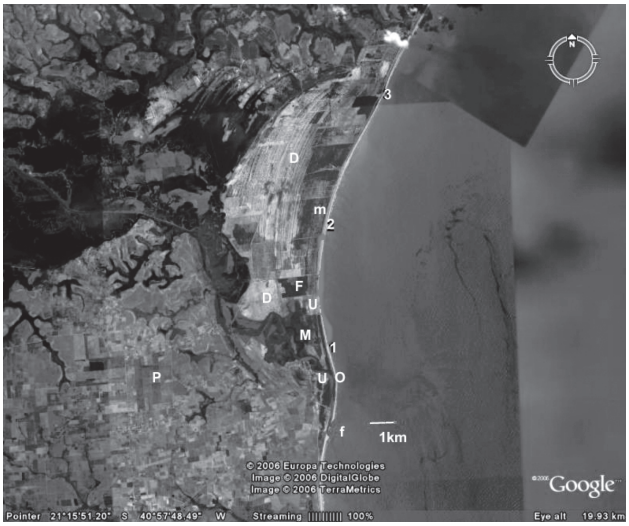

Figure 2. Praia das Neves beach in detail showing the distribution of the introduced population of *Liolaemus lutzae*. (O) Introduction site, (1-2) site of the major density of the introduced lizard population, (3) distribution extreme of the introduced species and site with the lowest density, (u) small urban area, (m) mangroove, (f) forest, (d) devasted area.

Table I. Morphological variation between two populations of the lizard *Liolaemus lutzae*.

Population	Snout-vent-lengh	Head-width
Barra de Maricá (1986)		
Number of cases (N)	47	47
Minimun (mm)	30.00	5.70
Maximum (mm)	78.60	12.70
Mean (mm)	56.48	9.72
Standard Deviation	14.33	2.10
Praia das Neves (2003)		
Number of cases (N)	138	137
Minimun (mm)	28.60	5.35
Maximum (mm)	84.25	15.80
Mean (mm)	52.88	9.07
Standard Deviation	12.78	1.95

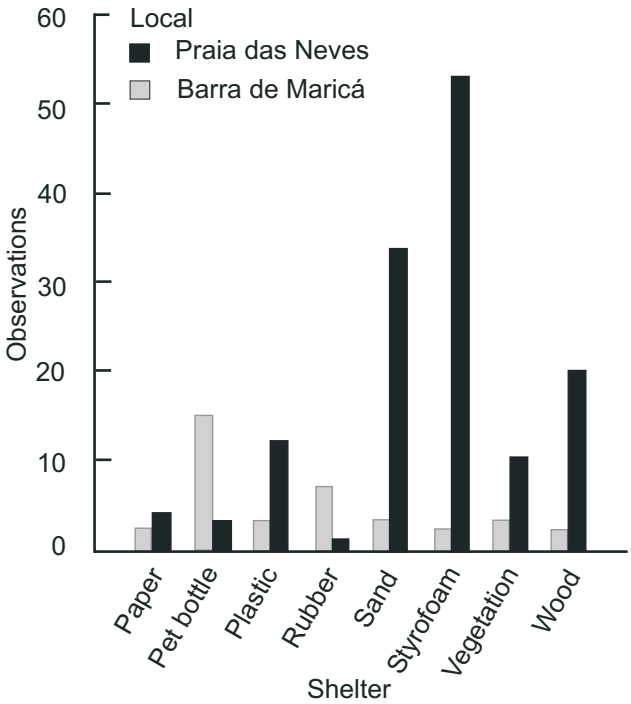

Figure 3. Differences in shelter use between the introduced and original populations of *Liolaemus lutzae*.

Table II. Number of items and volume (mm^3) of each prey type of *Liolaemus lutzae* in Praia das Neves (n = 37).

Categories	Number of itens	%	Volume	%
Vegetal material	57.00	17.38	7318.73	71.77
Hymenoptera	3.00	0.91	2.73	0.03
Formicidae	192.00	58.54	1011.43	9.92
Neuroptera	2.00	0.61	147.04	1.44
Coleoptera	24.00	7.32	584.66	5.73
Homoptera	32.00	9.76	78.76	0.77
Diptera	3.00	0.91	87.24	0.86
Orthoptera	3.00	0.91	95.21	0.93
Larvae	8.00	2.44	711.34	6.98
Aranae	4.00	1.22	144.58	1.42
Unidentified material	–	–	16.20	0.16
Total	328.00		10197.92	

Tropidurus torquatus (Wied, 1820), Iguanidae (n = 5), *Hemidactylus mabouia* (Moreau de Jonnés, 1818), Gekkonidae sp. (n = 3), *Mabuya agilis* (Raddi, 1823), Scincidae sp. (n = 1), and *Ameiva ameiva* (Linnaeus, 1758), Teiidae sp. (n = 1), all in low frequency if compared with the neighboring *restinga* shrub habitat (Costa *et al*. 1990) . In the beach habitat, we also found some known predators of *L. lutzae* (Rocha *et al*. 2000), like the crab *Ocypode quadrata* (Fabricius, 1787), Ocypodidae, the owl *Athene cunicularia* (Molina, 1782), Strigidae, the "Carcará" hawk, *Caracara plancus* (Miller, 1777), a species of Falconidae, and the colubrid snake, *Philodryas patagoniensis* (Girard, 1897).

Four females had eggs in their oviducts, four presented vitellogenic folicules in their ovaries, and seven were not reproductive. Only four out of 22 males were reproductive. Juveniles were more abundant in December, during the rainy season.

DISCUSSION

The translocated *L. lutzae* lizards formed a viable and reproductive population in the southern tip of Espírito Santo state, 200 km away from the original population (Barra de Maricá, Rio de Janeiro state). The white-sand-dune lizard space niche is conservative, when compared with the food niche. In Praia das Neves, *L. lutzae* are restricted to the beach, the species' habitat in Barra de Maricá, but showed a small change in diet, when compared with published data from Barra de Maricá (Rocha 1989).

The success of introduced populations depends on several factors, including adaptative responses to the new environment (Allendorf & Lundquist 2003, Sakai *et al*. 2001). The success also depends on the interactions between invaders and residents and studies have demonstrated that residents frequently dominate alien species (Massot *et al*. 1994, Myers *et al*. 2000b). In Praia das Neves, *L. lutzae* is restricted to strips of sand that can reach 50ºC in the warmest hours of the day. This microhabitat is probably inadequate for most lizard species in the community. Before the introduction of *L. lutzae*, *T. torquatus*, *A. ameiva*, *H. mabouia* and *M. agilis* were present in this habitat, in very few numbers, as observed by Costa *et al*. (1990). These lizard species still use the beach occasionally. On the other hand, no *L. lutzae* were found in the neighboring scrub habitat, suggesting that they are not spreading into the scrub habitats. However, since 2007 a monitoring study is being carried out by A.F.B. Araujo and colleagues. The program, financed by the FNMA (Fundo Nacional do Meio Ambiente - National Environmental Fund), aims at studying the interactions between *L. lutzae* and the original local fauna and flora, and the viability of management strategies (whether to remove or not the introduced lizard species).

Differences in body size among lizards from Barra de Maricá and the new population from Praia das Neves beach were expected as a result of the segregation of a small portion of the Barra de Maricá population in Praia das Neves. The founder event, associated with high levels of endogamy, genetic drift and geographic isolation, can lead to morphological differentiation (Grant *et al*. 2001). Phillips & Shine (2005) described morphological change resulting from the colonization of the toad, *Bufo marinus* (Linnaeus 1758), Bufonidae, in Australia. In reptiles, body size (and other life-history traits) varies

depending on humidity, temperature, and food supply (Van Damme *et al*. 1986) and can be understood as an adaptative strategy to cope with environmental variation (Massot *et al*. 1994). Our results did not reveal morphological differences between the two localities, refuting the hypothesis of strong morphological shift for this experiment.

Population differentiation can result from two different processes: the ability to adjust to changes in the environment over time (plasticity) and the capacity to adapt to a new environment (selection). The absence of significant morphological differences between the two populations in our study could be explained by the similarity among the two areas, in terms of environmental variability.

In both sites specimens were found under vegetation cover, but debris left by man, such as plastics, styrofoam, and industrial rubber, were also frequently used as shelter. In Barra de Maricá, plastic bottles were used more often, probably because the proximity (40 km) to Rio de Janeiro and Niterói cities (20 km) means abundant garbage. Trash from these cities is thrown into the sea, and ocean currents take it north to Barra de Maricá. It could be that the detritus deposited in Maricá helped *L. lutzae* to survive, since it is used by the species as a refuge and a feeding site. On the other hand, trash accumulation can endanger the vegetation, which in turn can compromise the survival of *L. lutzae*, that also uses plant as food and shelter.

Diet composition of *L. lutzae* differs between sites. In Barra de Maricá the lizard consumes more prey types. Both populations present an omnivorous diet, and plant material was the most prevalent diet item. Formicidae also constitute an important prey type. The observed difference between sites is due to elements such as Isopoda and Thysanoptera, present only in the Barra de Maricá sample.

Our results showed that plant material is a common food item and that the beach vegetation species are very important to the white-sand-dune lizard. Rocha (1989, 1998) Rocha *et al*. 2000) showed that *L. lutzae* may select and eat some plant species because of their water content, specially *Ipomoea littoralis* flowers Blume 1825, Convolvulaceae. This plant species is easily found on the beach at Praia das Neves. The leaf is eaten by *L. lutzae* and the scrubs are used as shelter.

Similarities (in animal and plant communities) between Praia das Neves beach and the original environment of *L. lutzae* may have helped the establishment of the introduced population. On the other hand, size of the founder population in Praia das Neves beach (51 individuals) could have hampered its establishment. Small isolated populations are more vulnerable because they loose genetic diversity due to genetic drift and endogamic depression (Simberloff 1988, Boyce 1992, Cornuet & Luikart 1996, Akçakaya & Sjogren-Gulve 2000, Lambert *et al*. 2005). Nevertheless, some vertebrate species seem to tolerate and remain unaffected by periods of small population size. Cheetah (O'Brien *et al*. 1983) suffered severe constraints and yet was able to survive. The large population of the lizard, *Tupinambis merianae* (Duméril & Bibron, 1839), Teiidae, in Fernando de Noronha Islands, in northern Brazil, resulted from the introduction of only two couples in 1960. Today, due to lack of predators, the population is firmly established and is considered a threat to the island ecosystems (the FNMA/IBAMA management program removed some individuals from the islands).

The area colonized by *L. lutzae* is specific, close to the sea, and other four lizard species are rarely observed (they can be seen in the neighboring scrub habitat). At the beach, *T. torquatus* is the most probable competitor of *L. lutzae*, both for food and space resources. This lizard species is the most abundant lizard population in the scrubs (Araujo 1991, Carvalho *et al*. 2007a), but it is very scarce at Praia das Neves beach.

Hemidactylus mabouia, an exotic gekkonid species, is also observed in the beach, but rarely. This lizard was probably brought in from Africa (Vanzolini 1978, Carranza & Arnold 2006) and is commonly found in bromeliads and dead trunks of the *restinga* scrub habitats (Costa *et al*. 1990, Araujo 1991, Carvalho & Araujo 2007, Carvalho *et al*. 2007b). This species coexists with *Gymnodactylus darwinii* (Gray, 1845), an endemic gekkonid of the Atlantic Forest biome, in the scrubs and also in the forest fragments. Considering that they share the same microhabitats in Praia das Neves, the resident species might be in danger, but until now there is no evidence the *H. maboia* has displaced any local species.

Introductions can be very dangerous when carried out without control. Experiments with the introduction of lizards on islands showed that Araneae communities can be affected (Schoener & Spiller 1996). Frits & Rodda (1998) demonstrated the impacts of the introduction of the snake *Boiga irregularis* Merrem, 1802, Colubridae, on the Island of Guam in 1952, where the snake led to the reduction and extinction of small vertebrates. These are examples in which the new population was introduced on islands, in restricted environments, and where the local fauna evolved in isolation. In such cases, ecological impacts can be more pronounced (Schoener & Spiller 1996).

Given the time elapsed since colonization and the lizard dispersal in the environment, we consider that the new colony has been firmly established. However, our observations do not support the hypothesis that this species will spread to the neighboring scrub habitats. The white-sand-dune lizard is restricted to beaches, a conservative feature shared with other sand lizard species of this genus in Brazil (Schulte *et al*. 2000). The distribution of other *Liolaemus* species living in Brazil, *Liolaemus occipitalis* Boulenger, 1885, and *Liolaemus arambarensis* Verrastro, Veronese, Bujes & Dias, 2003 is also restricted to beach habitats (Etheridge 2000).

Vanzolini & Ab'Saber (1968) suggested that living species of *Liolaemus* along the Brazilian coast descend from an extensively distributed single ancestral species vulnerable to the marine transgressions in the Quaternary. During the transgressions, many beaches disappeared and *restinga* areas diminished. As a result, a once continuous population fragmented into iso-

lated populations, by sea level variation promoted speciation events that generated current Brazilian sand lizard species. It is even possible that the *L. lutzae* ancestor was once more prevalent along the coast than is presently known. On the other hand, if dispersion was important to *L. lutzae*, as in the history of some groups of *Liolaemus* species (Morando *et al*. 2004), the mouth of Paraiba do Sul river, on the northern coast of Rio de Janeiro state, may have acted as a strong barrier, restricting dispersion further north along the coast.

Years after the *L. lutzae* introduction in Espírito Santo state, the IUCN published guidelines to restrict species translocation (Iucn 1994), due to the impacts caused by biological introductions. Considering these guidelines, species translocations can only be justified when there is a strong threat of extinction, and, even then, it should be the last alternative. We believe this is the case of *L. lutzae*. Firstly, the white-sand-dune lizard is extremely threatened by habitat reduction. For example, a resort called "Fazenda de São Bento da Lagoa", promoted by the European IDB group and the mayor of the Maricá municipality, will be built in the area that has the most dense and studied population of this lizard at the Barra de Maricá *restinga*. Secondly, in the state of Rio de Janeiro, the federal reservation that preserves *restinga* habitats, the Parque Nacional de Jurubatiba (Macaé), does not protect natural populations of the white-sand-dune lizard because they are located beyond the northern boundary of the *L. lutzae* distribution. Because experimental translocations are not easily found in the literature and alternative strategies do exist, it is vital that there be a continuous monitoring of the results of this management method (Dickinson & Fa 2000, Losos *et al*. 2001, Knapp & Malone 2003).

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