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PHYLOGEOGRAPHY, ECOLOGY AND CONSERVATION OF SKINK ADAMASTOR, *TRACHYLEPIS ADAMASTOR* CERÍACO, 2015 |

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FILOGEOGRAFIA, ECOLOGIA E CONSERVAÇÃO DA LAGARTIXA ADAMASTOR, *TRACHYLEPIS ADAMASTOR* CERÍACO, 2015

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Aos meus adorados Pais

Pedro (in memory and heart) e Teresa

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"Não é o mais forte que sobrevive, nem o mais inteligente, mas o que melhor se adapta às mudanças!" Charles Darwin

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Phylogeography, Ecology and Conservation of Skink Adamastor, *Trachylepis adamastor* Ceríaco, 2015

Abstract

The genus *Trachylepis* is one of the most diverse of reptiles inhabiting the Gulf of Guinea islands. *Trachylepis adamastor,* endemic to Tinhosa Grande islet, was recently described based solely on morphology. The phylogenetic relation between *T. adamastor* and remaining *Trachylepis* species, ecology and conservation status were unknown. This study investigates the phylogenetic and phylogeographic relationships, as well as the population density and the diet of *T. adamastor*. Results show that *T. principensis* from Príncipe and *T. adamastor* are genetically conspecific, although, given morphological differences and isolation, it is suggested that each population should be considered subspecies. The population density of *T. a. adamastor* was estimated as 0.012 per m^2 with 2460 individuals. These obtained values, together with its distribution makes *T. a. adamastor* a "Critically Endangered" subspecies according to IUCN. The diet of *T. a. adamastor* was also compared with that of the populations of the surrounding islands.

Filogeografia, Ecologia e Conservação da lagartixa Adamastor, *Trachylepis adamastor* Ceríaco, 2015

Resumo

O género de répteis *Trachylepis* é um dos mais diversos das ilhas do Golfo da Guiné. *Trachylepis adamastor,* endémica do ilhéu da Tinhosa Grande, foi recentemente descrita com base apenas na morfologia. A relação filogenética entre *T. adamastor* e as restantes espécies *Trachylepis*, assim como a sua ecologia e estatuto de conservação são desconhecidos. Este estudo investiga as relações filogenética e filogeográfica, densidade populacional e dieta de *T. adamastor*. Os resultados mostraram que *T. principensis* do Príncipe e *T. adamastor* são geneticamente coespecificas. Tendo em conta as diferenças morfológicas e o seu isolamento, é sugerido que cada população deve ser considerada como subespécie. A estimativa da densidade populacional obtida foi 0.012 por m^2 com 2460 indivíduos. Estes valores obtidos, juntamente com a distribuição da subespécie, tornam *T. a. adamastor* "Criticamente Em Perigo" de acordo com a IUCN. A sua dieta também foi comparada com a das populações das ilhas vizinhas.

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Main Introduction

Islands have always fascinated scientists (MacArthur & Wilson 1963; Foster 1964; MacArthur *et al.* 1972; Carlquist 1974; Hooijer 1976; Sondaar 1977; Heaney 1978; Báez 1982a,b; Reyment 1983; Lomolino 1983; Brown & Lomolino 1998; Grant 1998, 1998a; Whittaker 1998; Brown & Lomolino 2000; Calsbeek & Smith 2003; Vitousek *et al.* 2013; Cox *et al.* 2016). Their uncommon fauna and flora was used to establish both Darwin (1845) and Wallace (1902) theories, and since then island systems have become essential for studies of ecology and evolution (MacArthur & Wilson 1967; Whittaker 1998; Whittaker & Fernández-Palacios 2007; Lomolino *et al.* 2010). Their ecosystems are considered relatively simple which make them attractive for studies of ecology and evolution due to fewer, simpler, or stronger selective pressures (Whittaker 1998; Barahona *et al.* 2000; Schluter 2001). Theories such as island biogeography, dynamic equilibrium, and speciation, by MacArthur & Wilson (1967), illustrate the importance of studying islands. Besides that, islands also have importance at the biogeographic and conservationist level, owing to the high number of endangered or extinct species, and high number of endemism due to the processes of speciation (MacArthur & Wilson 1967; Mayr 1967; Whittaker & Fernández-Palacios 2007). Furthermore, islands are an excellent contribution to the study of biodiversity due to their peculiar characteristics (Adsersen 1995), such as the constitution of their communities, which are generally disharmonious given the absence of some taxa (Báez 1993; Cox & Moore 1993; Eliasson 1995; Itow 2003), the high number of endemism and different morphological characteristics such as dwarfism or gigantism.

In recent years the islands of the Gulf of Guinea have started to caught the attention of worldwide scientists. The Gulf of Guinea is located on the African coast and is composed of the continental island Bioko (also known as Fernando Pó), and the oceanic islands Príncipe, São Tomé and Annobon (also known as Pagalu) (Burke 2001). The Gulf of Guinea's islands are part of the Cameroon volcanic line, extending from Mount Cameroon (Cameroon, Africa) to Annobon island, having an extension of at least 1600 km (Lee *et al.* 1994). Except for Bioko, all other islands are volcanic in origin and were formed at different times, with Príncipe being the oldest, formed 31 My ago, followed by São Tomé 14 My ago and Annobon 4.8 My (Lee *et al.* 1994). Bioko, separated from the African continent, was isolated due to sea level rise only 10-11 000 years ago (Eisentraut 1965; Moreau 1966). The three oceanic islands have never been

linked to the mainland (Jones 1994; Lee *et al.* 1994), and these islands are isolated by seawaters with depths over 4000 m (Jones 1994; Marzoli *et al.* 2000). Bioko differs from the previous three islands since it lies on the continental shelf, with depths of only 60 m, it is the largest island with 2017 km^2 and the nearest to the mainland, by only 32 km (Lee *et al.* 1994; Marzoli *et al.* 2000). The island of São Tomé has an area of around 836 km^2 and is about 280 km from the African continent (Lee *et al.* 1994; Marzoli *et al.* 2000). The Príncipe is smaller, having an area of around 128 km^2 and is located closer to the mainland, about 250 km (Lee *et al.* 1994; Marzoli *et al.* 2000). Annobon is the smallest of these four islands, with only 17 km^2, and is the farthest from the African continent, about 375 km (Lee *et al.* 1994; Marzoli *et al.* 2000).

According to WWF (2017), the islands of São Tomé, Príncipe and Annobon are an Afrotropical ecoregion and have a vulnerable status. Biogeographically the Gulf of Guinea islands belongs to the rainforest zone of West Africa, being located between two large regions - Guinean forest and Congo basin. These islands have recently received more attention due to their exceptional biodiversity and are part of one of the largest biodiversity hotspots in the world (Myers *et al.* 2000; Drewes 2002; Measey *et al.* 2007; Jesus *et al.* 2009). The geographic characteristics and the isolation of the islands gave origin to rapid speciation processes in its fauna, leading to a high rate of endemism (Frade 1958; Dutton 1994; Jones 1994; Drewes 2002; Measey *et al.* 2007; Uyeda *et al.* 2007; Vaz & Oliveira 2007; Melo *et al.* 2011).

Despite their relatively small size, the islands present a considerable diversity of flora and fauna species and exhibits one of the greatest number of endemic species per area worldwide (Juste 1996; Drewes & Wilkinson 2004). According to Exell (1973), the number of known flora species, including those introduced and non natives, in the Príncipe are 314 and 601 in São Tomé. The rates of plant endemism for Príncipe is 9.9% and for São Tomé it is 7.7% (Exell 1973). At the national level, the flora includes 157 Pteridophyta (ferns) and 791 plants with seeds (spermatophyte) (Figueiredo 1994; Vaz & Oliveira 2007). Orchids, which have 135 species, being 35 endemics, are an example of one particularly rich group (Lejoly 1995; Vaz & Oliveira 2007). The native fauna of Príncipe includes four species of bats, one of shrew (Feiler 1988; Feiler *et al.* 1993; Vaz & Oliveira 2007), ten of reptiles and three of amphibians (Ceríaco *et al.* in press). São Tomé included nine species of bats, one of shrew (Feiler 1988; Feiler *et al.* 1993; Vaz & Oliveira 2007), ten of reptiles and five of amphibians (Ceríaco *et al.* in

press) as native species. In addition to these, the remaining mammals found on the islands are practically all introduced, such as monkeys, rats, weasels, cats, dogs, etc. (Feiler 1988; Feiler *et al.* 1993; Dutton 1994). The rate of amphibian endemism in the São Tomé and Príncipe is 100% (Ceríaco *et al.* in press). For the reptiles, with the exceptions of *Pelusios castaneus* (Schweigger, 1812) in São Tomé, *Trachylepis affinis* (Gray, 1838) in Príncipe, and *Hemidactylus mabouia* (Moreau de Jonnès, 1818) and *Hemidactylus longicephalus* Bocage, 1873 on both islands (Manaças 1958), all other reptile species appear to be endemic (Ceríaco *et al.* in press). It is worth noting the high endemism of terrestrial snails, with about 60 endemic species between São Tomé, Príncipe and Annobon (Angus 1994), and of butterflies with 64 in São Tomé and 45 in Príncipe (Pyrez 1992). Invertebrates also have a high rate of endemism in both islands (Mendes & Bívar-de-Sousa 2012). In the case of marine reptiles, these islands have importance as a nesting site for sea turtles (Graff 1996). Birds on the islands include 33 terrestrial species and at least 6 species of seabirds in Príncipe and 51 terrestrial and freshwater species and 6 seabirds in São Tomé (Jones & Tye 2006; Leventis & Olmos 2009; Valle *et al.* 2014; Lima *et al.* 2016).

Since the end of the 15th century, the country's biodiversity has always been disturbed by anthropogenic activities, particularly in the lower altitude areas that have been occupied by sugarcane, coffee and cocoa plantations (Lains & Silva 1958) and numerous exotic species were introduced (Feiler 1988; Feiler *et al.* 1993; Dutton 1994). In recent years, there has been an impoverishment of the biological diversity of the archipelago's ecosystems, which translates into a deterioration in the quality of life of the populations that depend on them (Vaz & Oliveira 2007). Due to its high number of endemisms and taxonomic diversity, São Tomé and Príncipe forests are a global priority for conservation groups (Jones 1994; Juste & Fa 1994). These forests are included in the list of sites under imminent threat of extinction of Alliance for Zero Extinctions (American Bird Conservancy 2017) and they are considered the second highest priority site for bird conservation in Africa, as "conservation hotspots" and priority ecoregions (Leventis & Olmos 2009).

During the last decade these islands have received a lot of attention by international researchers, leading to a large number of published studies (Drewes & Stoelting 2004; Jesus *et al.* 2005a,b, 2006; Jones & Tye 2006; Measey *et al.* 2007, Jesus *et al.* 2007, 2009; Dallimer *et al.* 2009; Melo *et al.* 2011; Valle *et al.* 2014; Lima *et al.*

2015, 2016), and descriptions of new species of vertebrates (Measey *et al.* 2007; Uyeda *et al.* 2007; Miller *et al.* 2012; Ceríaco 2015; Ceríaco *et al.* 2015; Bell 2016; Ceríaco *et al.* 2016). Habitat destruction, introduction of exotic species and hunting are the main threats of São Tomé and Príncipe. Despite having a Law on the Conservation of Fauna, Flora and Protected Areas (Law n. 11/99), it still needs to be properly implemented (Vaz & Oliveira 2007). Obô Natural Park of São Tomé and Príncipe, with 295 km of area and that was created in 1993 but was only made official in 2006 (DGA 2006). In 2011, Príncipe Island was classified by UNESCO as World Biosphere Reserve.

The islands of São Tomé and Príncipe are surrounded by islets and rocks of diverse sizes. Closer to São Tomé are the Rolas Islet and the Cabras Islet, larger islets with vegetation. Others, such as the Sete Pedras, on the south coast of São Tomé, and the Tinhosas, south of the Príncipe, mainly exhibit exposed rocks and only a few scattered herbaceous plants (Leventis & Olmos 2009). The Tinhosas (Fig. 1, 2), located approximately 20 km southwest of the Príncipe island are two small rocky islets, the Tinhosa Grande (N: -1,34135556, E: -7,29151389; WGS84) and the Tinhosa Pequena (N: -1,38241667, E: -7,28316944, WGS-84). Tinhosa Grande (Fig. 3) has a surface area of around 20.5 ha, while Tinhosa Pequena has only 3.3 ha (Leventis & Olmos 2009).
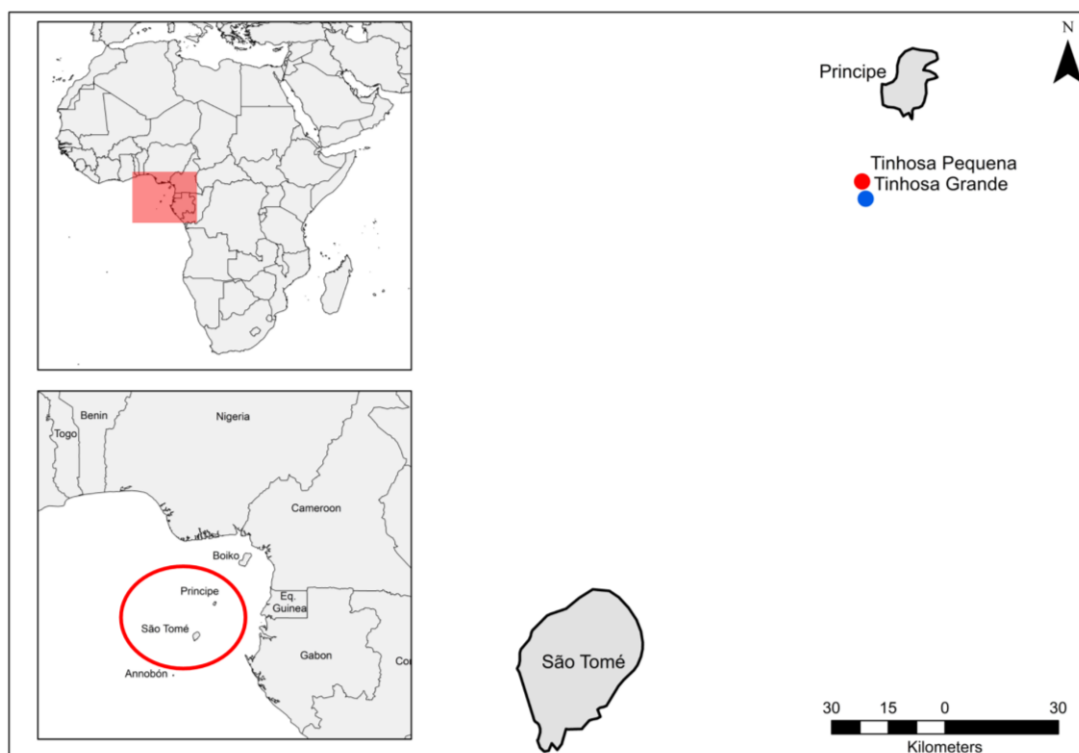
FIGURE 1. Geographical location of São Tomé and Príncipe islands and Tinhosas islets.

FIGURE 2. Tinhosa Grande and Tinhosa Pequena islets aerial photo (Photo: Luis Ceríaco).

FIGURE 3. Tinhosa Grande islet view from the boat (Photo: Ana Carolina Sousa).

Both islets are classified as Important Bird Areas (IBA) (BirdLife International 2017). They were also recognized as Wetlands of International Importance and official Waterfowl Habitat (RAMSAR 2017). They are large bare boulders devoid of any vegetation and feature some of the most important seabird colonies in West Africa, with tens of thousands of breeding pairs that use the place to nest (Jones & Tye 2006) (Fig. 4). The 4 species of seabirds that nest here are Brown Noddy, *Anous stolidus* (Linnaeus, 1758)*,* Black Noddy, *Anous minutus* Boie, 1844*,* Brown Booby, *Sula leucogaster* (Boddaert, 1783), and Sooty Tern, *Onychoprion fuscatus* (Linnaeus, 1766) (Leventis & Olmos 2009; Valle *et al.* 2014; BirdLife International 2017). In an islet with such a small area and harsh environment, the presence of terrestrial vertebrates was not expected. However, two species of reptiles are found on the Tinhosa Grande islet – a gecko (Family Gekkonidae) (Fig. 5) and a skink (Family Scincidae) (Ceríaco 2015).

FIGURE 4. Tinhosa Grande islet with tens of thousands seabirds of breeding pairs (Photo: Luis Ceríaco).

FIGURE 5. *Hemidactylus* sp. in life from Tinhosa Grande islet (Photo: Luis Ceríaco).

The family Scincidae (Sauria: Squamata) has about 1,613 species, being one of the largest families of lizards in the world (Uetz & Hošek 2016). Scincids lizards exhibit a high diversity of body shapes, sizes and habits. They range from normal-legged animals, through tiny-legged to legless species, from very diminute species to considerably large animals, and can be semi-fossorial, the forest and savanna dwellers, leaf litter specialists, or sandy deserts inhabitants (Trape *et al.* 2012; Speybroeck *et al.* 2016). There are several genera of skinks on the oceanic islands of Gulf of Guinea as the case of *Trachylepis* Fitzinger, 1843; *Feylinia* Gray, 1845 and *Panaspis* Cope, 1868 (Uetz & Hošek 2016).

The genus *Trachylepis* Fitzinger, 1843 currently contains about 78 species (Uetz & Hošek 2016), with new species constantly being described, such as *T. gonwouoi,* recently described by Allen *et al.* (2017). *Trachylepis* is one of the most speciose genera of skinks (Uetz & Hošek 2016). It is distributed throughout Africa, on the islands of Comores, Madagascar, Seychelles, and also in Fernando Noronha island off the coast of Brazil, being one of the richest genus in terms of lizard species in the Gulf of Guinea islands (Mausfeld *et al.* 2000; Bauer 2003; Uetz & Hošek 2016). Five species of *Trachylepis* are known for the oceanic islands of the Gulf of Guinea, some of them recently described: the endemic *Trachylepis thomensis* Ceríaco, Marques & Bauer,

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2016, on São Tomé island and the Rolas Islet; the endemic *Trachylepis principensis* Ceríaco, Marques & Bauer, 2016, on Príncipe island (both previously considered insular populations of *Trachylepis maculilabris* Gray, 1845) (Ceríaco *et al.* 2016); *T. adamastor* Ceríaco, 2015, endemic of Tinhosa Grande islet off the coast of Príncipe (Ceríaco 2015); *T. affinis* (Gray, 1838) on Príncipe island; and *T. ozorii* (Bocage, 1893) endemic to Annobon island (Manaças 1958, 1973; Jesus *et al.* 2003, 2005b).

In the last decades, vertebrate species description has seen a steady increase in many groups (Costello *et al.* 2013; Fjeldså 2013). Reptiles are one of the vertebrate group with the highest species rate found globally (Pincheira-Donoso *et al.* 2013), with 10,450 species described as of July 2017 (Uetz & Hošek 2016). Although hundreds of reptile species are described per year (Uetz & Hošek 2016), this group is poorly represented on The IUCN Red List of Threatened Species[TM], with only 52,37% of described species evaluated (IUCN 2017). Böhm *et al.* (2013) analyzed for the first time globally reptile conservation status. The global extinction risk of reptiles was analyzed with the selection of only 1500 random species (just 14,4% of the 10,450 described species as of July 2017; Uetz & Hošek 2016) being many of these (21%) classified as "Data Deficient" (Böhm *et al.* 2013). The high percentage of "Data Deficient" taxa can bring many constraints. For example, the risk of extinction is negatively affected when "Data Deficient" species are not considered (Butchart & Bird 2010, Hoffmann *et al.* 2010; Bland *et al.* 2012), thus affecting conservation priorities (Brooks *et al.* 2006). In addition, species classified as "Data Deficient" are rarely included in legislation or conservation plans (Sousa-Baena *et al.* 2014). The low number of reptile species evaluated can possibly be explained by the lower level of research on these group (Bonnet *et al.* 2002a), their secretive behavior (Doody *et al.* 2013), high rates of cryptic species diversity (Oliver *et al.* 2009; Rosauer *et al.* 2016) and public aversion (Keller 1993). There are still many reptile species to be described, and Meiri (2016) predicts that Africa will be the hotspot for future species descriptions. For these reasons, studies like the present are essential, since only what is known can be preserved.

Trachylepis adamastor (Fig. 6) was recently described by Ceríaco (2015) based on eight specimens from the collections of the Instituto de Investigação Científica e Tropical (IICT), collected in the early 1970's in Tinhosa Grande islet. According to the author, the newly described species could be easily distinguished from all other *Trachylepis* species by differences in color, size and lepidosis – large and robust body

size, up to at least 112.0 mm (SVL); dorsal brown-dark coloration, with subtle black and white speckles in the dorsum and a grayish venter; 31-34 midbody scale rows, 49-54 longitudinal dorsal scales, 63-66 scales across the venter, 5 to 6 keels in the dorsal, smooth scales on sole of feet and hands, having only one pretemporal scale and very small ear opening.

FIGURE 6. *Trachylepis adamastor* in life from Tinhosa Grande islet (Photo: Luis Ceríaco).

Due to the presence of some synapomorphies, Ceríaco (2015) suggested that *T. adamastor* belongs to the same evolutionary lineage of the complex *T. maculilabris* existing in São Tomé and Príncipe - *T. thomensis* and *T. principensis,* respectively. There are several examples in the Gulf of Guinea oceanic islands of amphibians (Drewes & Wilkinson 2004) and reptiles (Jesus *et al*. 2007) species who colonized the different island through "island hopping", so it is possible that this is a similar case. However, according to Ceríaco (2015) it is still necessary to analyze the possibility of the isolation of this population due to changes in sea level that separated the island from Príncipe island, since there is evidence that Príncipe island has been much more extensive in the past.

T. adamastor is one of the larger species of the genus (Ceríaco 2015). Size related adaptations, as gigantism and dwarfism, are common in reptiles populations

inhabiting isolated areas such as oceanic islands (Case 1978; Andreone & Gavetti 1998; Andreone 2000; Barahona *et al.* 2000; Carranza *et al.* 2001; Filin & Ziv 2004; Keogh *et al.* 2005; Meik *et al.* 2010). In fact, the characteristic that most quickly tends to change on islands is the body size (Case 1978). Actually, according to Mertens (1934), some groups of lizards including Scincidae show a general trend toward larger body size on islands.

Foster (1964) was the first to quantify differences in body size between islands and mainland mammal's populations. Later, in 1973 van Valen interpreted his results and hypothesized whereby small species tendency toward larger body size and large species toward smaller body size and named "the island rule". Although some taxa do not follow this pattern (rodents, Lawlor 1982; mammalian carnivores, Meiri *et al.* 2004, 2006, 2008; ungulates, Raia & Meiri 2006; lizards, Meiri 2007; turtles, Itescu *et al.* 2014), many vertebrate taxa exhibit "the island rule" (birds, Clegg & Owens 2002; snakes, Boback & Guyer 2003; bats, Lomolino 2005; primates, Bromham & Cardillo 2007). In addition, this theory has been used to support evolutionary concepts such as optimal body size (Brown *et al.* 1993; Boback 2006). Most recently, Meiri *et al.* (2011) concluded that although mammals tend to follow the "island rule", birds and lizards did not show this trend in neither at a gender or family levels.

Despite the various assumptions about changing body size on islands, the most common explanations for these shifts in relation to gigantism are the relaxation of predation (Case 1978, 1982; Lomolino 1985; Grant 1998), such as the absence of mammalian predators (Szarski 1962; Case 1978, 1982; Pregill 1986; Greer 2001; Meiri 2008), competition pressures and large prey or abundant food sources (Case 1978, 1982; Lomolino 1985; Case & Schwaner 1993; Grant 1998; Lomolino 2005; Raia & Meiri 2006; Meiri 2007; Pafilis *et al.* 2009). In addition to these conjectures it is also said that the presence of seabirds conditions the density of populations and the body size of many vertebrates (Sanchez-Piñero & Polis 2000; Bonnet *et al.* 2002b) due to the accumulation of fecal matter, food remains and carcasses to increase the availability of nutrients in the islands (Polis & Hurd 1996). Thus, *T. adamastor* body size may be related to the presence of tens of thousands of seabirds on the islet.

According to Fisher & Owens (2004) and Meiri (2008), large body size animals are often associated with an increased risk of extinction. Considering the previous

aspects, body size studies are very important due to its influence on organisms morphology, physiology and ecology.

Many populations of squamate reptiles have distinct color morphs, being melanism the most frequent (Luiselli 1992; Forsman 1995). Melanism typically occurs with relatively high frequency in cooler climates, such as foggy coastlines or at higher elevations and latitudes, and on islands (Mouton & Oelofsen 1988; Badenhorst *et al.* 1992; Luiselli 1992; Ortiz 1994; Forsman 1995; Monney *et al.* 1995). Furthermore, Badenhorst (1990) established the relationship between lowland melanistic populations with zones of upwelling of cold water in the Atlantic Ocean. There have been several studies on evolutionary adaptations on color polymorphisms, especially on melanism (Norris & Lowe 1964; Wiens 1999; Cox & John-Alder 2005; Jansen van Rensburg *et al.* 2009).

Melanism has implicated several physiological functions such as: thermoregulation, dark animals are able to warm up faster and maintain higher body temperatures (Kettlewell 1973; Kingsolver & Wiernasz 1991; Vences *et al.* 2002; Clusella-Trullas *et al.* 2007); protection from ultraviolet (UV) radiation (Kollias *et al.* 1991; Setlow *et al.* 1993; Gunn 1998; Hofer & Mokri 2000; Callaghan *et al.* 2004; Calbó *et al.* 2005); immune function (Mackintosh 2001; Wilson *et al.* 2001); pleiotropic effect of selection on other functions of melanin, melanocortins, immunocompetence, stress resistance, and energy balance (Hoekstra 2006; Ducrest *et al.* 2008). Moreover, other functions such as cryptic coloration (Kettlewell 1973; Endler 1984), aposematism (Turner 1977), disease resistance (Wilson *et al.* 2001) and intraspecific communication (sexual selection) (Wiernasz 1989) are associated with melanism.

According to Bergmann's rule, it is expected that endothermic species inhabiting colder environments are generally larger than species inhabiting in warmer regions (Bergmann 1847; Blackburn *et al.* 1999). This rule was analyzed also in ectothermic species (Ray 1960; Lindsey 1966; Ashton & Feldman 2003; Olalla-Tárraga & Rodríguez 2007; Pincheira-Donoso & Meiri 2013), but had contradictory results by showing different trends among different groups.

There are two hypotheses which are complementary and explain the reason of larger ectotherms may occur in colder environments. The first is the thermal melanism hypothesis (TMH; Gates 1980), which predicts that darker animals may be favored

more than lighter animals because they potentially gain heat faster (Lusis 1961; Kettlewell 1973; Clusella-Trullas *et al.* 2007). The second, proposed by Olalla-Tárraga & Rodríguez (2007), is the "heat balance hypothesis" (HBH) for small ectotherms, predicts that larger animals will be favored in cold environments because of their greater heat conservation potential.

Although melanism has many functions, it may have some associated costs, such as a high risk of predation by being more visually exposed (Andrén & Nilson 1981). However, when dark animals inhabit environments with a dark background, such as lava flow, the risk of predation is lower when compared to colored animals, favoring the melanistic individuals (van Damme *et al.* 1989; Sinervo *et al.* 1991).

Considering the small size of the Tinhosa Grande islet, and consequently poor habitat and low food availability in relation to the larger islands or the mainland, it seems that *T. adamastor* experienced a radical adaptation. Its giant form may be related to the exposed environment, the lack of shelter and an adaptation to periods of low food (Ceríaco 2015). However, the scarcity of food and the adverse habitat conditions suggests that this species has an opportunistic and generalist diet. *T. adamastor* was also observed feeding on split eggs of nesting birds in Tinhosa Grande (Ceríaco 2015), which may corroborate this hypothesis. A study on the diet of another Scincid species (*Mabuya agilis*), in an insular habitat in Brazil, recorded 11 types of prey in their diet, characteristic of an opportunistic predator (Rocha *et al.* 2004), the same can happen with *T. adamastor*.

The description of *T. adamastor* is also intimately linked to museum collections. While the environment has been changing steadily over the last years, many species have become extinct due to climate change but also to anthropogenic activities (Rocha *et al.* 2014). Many authors have hypothetized that the collection of specimens for museums may also have been a threat to the world's biodiversity (Fuller 1999; Collar 2000; Hume & Walters 2012). It has been suggested that instead of collecting individuals, information should be collected through photographs, audio recordings, molecular data, and non-lethal tissue samples (Cheng *et al.* 2011; Minteer *et al.* 2014). However, the isolated application of these resources becomes problematic, and even when applied together they do not give us enough information to identify or describe a species or genus, both animal and plant (Rocha *et al.* 2014). Collecting is currently regulated and follows strict ethics guidelines, so that population demography is not

affected (Collar 2000; Winker *et al.* 2010). Museum specimens however play a much bigger role in conservation than usually is considered. A practical example corroborating that is the case of the negative effects of climate changes in animals, in which the reduction of the body size of these animals was only discovered through the analysis of the morphology of museum specimens (Gardner *et al.* 2011). Cheng *et al.* (2011) also shows the need to collect specimens for propagation studies of the chytrid fungus infection in amphibians. Porter & Wiemeyer (1969), through the thinning of egg shells from birds collected over a period have decided to ban DDT and other environmental pollutants. Another example that aims to prove that the collection of specimens is not invasive is the case of the endemic species *Micrathene whitneyi graysoni*, which was common when samples were collected between 1896 and 1932, and the most probable reasons for their extinction (in 1970), were habitat degradation and predation by invasive species, and not the collection of specimens made by humans as previously thought (Hume & Walters 2012). In addition to these examples, to estimate the risk of species extinction, especially for widely distributed species, the IUCN Red List criteria require specific and detailed information on life history and biology (such as longevity and growth rate), and for this the collection of species is crucial (Sadovy de Mitcheson *et al.* 2013).

Sometimes, the specimens are not collected for a particular purpose, and can be used in many ways by several scientists. Nowadays, with modern technologies, the biological collections are the most important for conservation, ecology and evolution studies (Bi *et al.* 2013). Furthermore, natural history collections contribute to taxonomic and community levels studies (Johnson *et al.* 2011) such as biogeographic range changes (Boakes *et al.* 2010), phenological shifts (Robbirt *et al.* 2011) and evolutionary change (Inger & Bearhop 2008). It is estimated that about 86% of the planet's species remain unknown (Mora *et al.* 2011). Thus, it is of extreme importance to document biodiversity as rigorously as possible, so that management and conservation decisions can be made both now and in the future. Yet, to do this it is vital that the collections and their samples are well planned and preserved (Winker *et al.* 2010; Rocha *et al.* 2014).

The case of *T. adamastor* is an excellent example that portrays the importance of museum collections. The description of this species was based on the eight specimens that were forgotten in the IICT. It is important that more attention be paid to collections like this to prevent extinction of species before we know them. The IICT which includes

the collection of this study is a very important repository of the biodiversity of Africa, since there are few collections in the world that include this area. It is essential to preserve unique collections of natural history of this type and not to let them be forgotten (Ceríaco 2015).

Due to its recent description, a lot is still unknown regarding *T. adamastor*, such its phylogenetic and phylogeographic placement within the genus, or its population density. Phylogeography is a growth field of evolutionary biology, interested in the study of genetic lineages with the purpose of perceiving the geographic and demographic history within and between closely related species (Avise 2009). This can be accomplished by genomic approaches through molecular markers, as mitochondrial or nuclear DNA, associated with morphological data (Brito & Edwards 2009; Portik & Bauer 2012), giving us important tools for the interpretation of current species distributions in an evolutionary context.

Reptiles have been used as model organisms for ecological and evolutionary studies from individual to community levels, and many species have been studied and incorporated into phylogeographic studies worldwide, being common in many regions of Europe (e.g. Harris & Sá-Sousa 2002; Podnar *et al.* 2005; Carranza *et al.* 2006; Vaconcelos *et al.* 2006; Ursenbacher *et al.* 2006; Ursenbacher *et al.* 2008; Fonseca *et al.* 2009), North America (e.g. Burbrink 2002; Wiens & Penkrot 2002; Feldman & Spicer 2006; Leaché & Mulcahy 2007), Australia (Rawlings & Donnellan 2003; Chapple & Keogh 2004; Dolman *et al.* 2006; Pepper *et al.* 2006; Hodges *et al.* 2007; Lukoschek *et al.* 2007; Moussalli *et al.* 2009), among others. Other countries with highly diverse herpetofauna as Africa remain poorly studied with the majority of studies only focused in areas within Southern Africa (Makokha 2006; Tolley *et al.* 2006; Daniels *et al.* 2007; Tolley *et al.* 2008; Portik & Bauer 2012; Swart *et al.* 2009).

The use of reptiles as models on phylogeographic studies, is partly explained due to their low to moderate levels of movement and dispersion when compared with vertebrate groups, such as birds and mammals, which help to maintain historical patterns (e.g. genetic and geological events). They also exhibit highly morphological and genetic variability, and are widely distributed geographically, occupying a wide range of habitats (Camargo *et al.* 2010) as well as easy to collect, promoting behavioral and ecological studies. Population density is one of the most helpful tool used for

innumerable ecological purposes (Efford & Fewster 2013) and it is of particular importance to conservation and management of populations.

The species richness is affected by habitat diversity (Williams 1964; Connor & McCoy 1979), more habitats in a given area will have more species inhabiting there. On islands a positive association between area and species richness has been noted (Preston 1948; Williams 1964; MacArthur 1972; Simberloff 1974; Williamson 1981, 1988; Rosenzweig 1995). However, population density of a species fluctuates depending on available resources and with the interactions with other species in the area where it inhabits (Damuth 1981, 1987; Tilman 1994). The environmental and ecological limitations equilibrium on population density are greatly changed on islands (Buckley & Jetz 2007). Population densities tend to be higher on islands species when compared with mainland (MacArthur *et al.* 1972; Rodda & Dean-Bradley 2002; Buckley & Jetz 2007), the lower number of species on small or isolated island may lead to this and to ecological release. MacArthur *et al.* (1972) reported that although birds have high population densities on islands, they exhibit lower species richness. This phenomenon is nominated density compensation, and has been well verified on lizards (Case 1975; Case & Bolger 1991; Rodda & Dean-Bradley 2002). Insular species may have an increasing population density due to the lower interspecific competition and predation risk (MacArthur *et al.* 1972; Adler & Levins 1994). Despite this, species may suffer variations in their real density due to environmental aspects and body size as example (MacArthur *et al.* 1972; Novosolov *et al.* 2013). Numerous studies support density compensation and the high population densities on islands (Buckley & Jetz 2007; Novosolov *et al.* 2013). Knowing the abundance of animals is crucial to understand their ecology, nevertheless, many estimates of population density are untrustworthy because they are not representative (Rodda *et al.* 2001). Population density has to be evaluated mostly indirectly, since the direct and exhaustive counting of animals is very difficult and sometimes impossible (Efford & Fewster 2013). The lizards perform a relevant component in food chain in several environments, thus, many ecological studies included this group as a model, especially on islands. There are some estimate population density studies with lizards (e.g. Ruibal & Philibosian 1974; Gorman & Harwood 1977; Bullock & Evans 1990; Rodda *et al.* 2001; Pérez-Mellado *et al.* 2008; Efford & Fewster 2013; Anton *et al.* 2014; Novosolov *et al.* 2015) for different species and purposes.

Main Objectives

The only available exemplars of *T. adamastor* correspond to eight specimens from the collection of IICT, from early 1970's. Despite the available molecular data between the *Trachylepis* species of the Gulf of Guinea there is no information regarding *T. adamastor*. Only recent morphological data where published regarding *T. adamastor*. Considering the current knowledge of *T. adamastor*, and the lack of information about their relationship with *T. principensis* and *T. thomensis*, and their morphological differences (size, coloration and scale numbers), as well the Tinhosa Grande islet conditions (isolation, habitat and limited area), this dissertation aims to provide the first genetic and ecological (population density and diet) data to the scientific community, in order to try to understand the phylogenetic relationships between the three species, the ecological habits of *T. adamastor*, as well the most effective conservation strategies to be applied.

Given that, it is expected to: 1) Provide the first study the phylogenetic and phylogeographic relationships of *Trachylepis adamastor;* 2) Estimate the population density of *Trachylepis adamastor* in Tinhosa Grande islet; 3) Understand the trophic ecology and ecological relations of *Trachylepis adamastor*.

To achieve the proposed objectives, an expedition was conducted in São Tomé and Príncipe islands and Tinhosa Grande islet, with the intention of collecting new fresh material to combine with the available data found at museum collections to analyze and compare all material though molecular, morphological and ecological methods.

Paper I: Phylogeography of the Adamastor skink *Trachylepis adamastor* on the Tinhosa Grande islet, Gulf of Guinea.

Abstract

Six species of the genus *Trachylepis* occur in the Gulf of Guinea - *T. maculilabris*, *T. ozorii*, *T. affinis, T. thomensis, T. principensis and T. adamastor*. This paper aims to present the first phylogenetic and phylogeographic analysis regarding the position of *T. adamastor* among its congeners. Morphological data show striking phenotypic differences between *T. adamastor*, *T. thomensis* and *T. principensis*, such as body size and coloration. Although phylogenetic analyzes using the 16S and ND2 mitochondrial genes and the RAG-1 nuclear gene, showed that the species *T. principensis* and *T. adamastor* are genetically conspecific. This study discusses the taxonomic and nomenclatural implications of these findings, analyze the different taxonomic approaches, and review the impact that the potential taxonomical rearrangement may have on conservation.

Key words: São Tomé & Príncipe, Tinhosa Grande, *Trachylepis adamastor*, *Trachylepis principensis*, Phylogeny, Phylogeography, Taxonomy.

Resumo

São atualmente conhecidas para o Golfo da Guiné seis espécies do género *Trachylepis* - *T. maculilabris, T. ozorii, T. affinis, T. thomensis, T. principensis e T. adamastor*. Este artigo pretende fornecer a primeira análise filogenética e filogeográfica da espécie *T. adamastor* e dos seus congéneres. Os dados morfológicos exibem diferenças fenotípicas muito acentuadas entre a espécie *T. adamastor* e as espécies *T. thomensis* e *T. principensis*, como por exemplo no tamanho do corpo e na coloração. Apesar disto, através de análises filogenéticas utilizando os genes mitocondriais 16S e ND2 e o gene nuclear RAG-1, observou-se que as espécies *T. principensis* e a *T. adamastor* são coespecíficas. Neste estudo discutem-se as implicações taxonómicas e nomenclaturais destes resultados, ao mesmo tempo que se analisam as diferentes possíveis abordagens taxonómicas, e o seu potencial impacto para a conservação.

Palavras chave: São Tomé e Príncipe, Tinhosa Grande, *Trachylepis adamastor*, *Trachylepis principensis,* Filogenia, Filogeografia, Taxonomia.

Introduction

Trachylepis Fitzinger, 1843 is one of the most speciose genera of scincidae and currently contains about 78 species (Uetz & Hošek 2016), however new species are constantly being described, as is the case of *T. gonwouoi* described in May 2017 (Allen *et al.* 2017). The genus *Trachylepis* have a wide distribution throughout Africa, from the mainland, to the islands of the Comores, Madagascar, Seychelles, Gulf of Guinea, and Fernando Noronha, an archipelago in Brazil (Mausfeld *et al.* 2000; Bauer 2003; Uetz & Hošek 2016). In the Gulf of Guinea oceanic islands, the genus is one of the richest in terms of species diversity, with five *Trachylepis* species inhabiting on these islands: the endemic *Trachylepis thomensis* Ceríaco, Marques & Bauer, 2016, on São Tomé island and Rolas Islet (Ceríaco *et al.* 2016); *Trachylepis principensis* Ceríaco, Marques & Bauer, 2016, endemic to Príncipe island (Ceríaco *et al.* 2016); *T. adamastor* Ceríaco, 2015, endemic to Tinhosa Grande islet off the coast of Príncipe (Ceríaco 2015); *T. affinis* (Gray, 1838) on Príncipe island; and the *T. ozorii* (Bocage, 1893) endemic to Annobon island (Manaças 1958, 1973; Jesus *et al.* 2003, 2005a).

The islands of the Gulf of Guinea are one of the biodiversity hotspots of the world, biogeographically part of the rainforest zone of West Africa (Myers *et al.* 2000; Drewes 2002; Measey *et al.* 2007; Jesus *et al.* 2009). They are a part of the Cameroon volcanic line, having an extension of at least 1600 km (Lee *et al.* 1994). Bioko is the largest island (2017 km^2) and the only one that has ever been linked to the mainland, being the one closest to the African continent, by only 32 km (Lee *et al.* 1994; Marzoli *et al.* 2000). The other three islands, all of them oceanic, have never been connected to the mainland, have volcanic origin, and were formed at different years. Príncipe is the oldest, formed 31 My ago, followed by São Tomé 14 My ago and finally the most recent Annobon 4.8 My ago (Lee *et al.* 1994). Príncipe (128 km^2) is the closest island to the African continent, only 250 km away, followed by São Tomé (836 km^2) at 280 km and Annobon (17 km^2) at 375 km (Lee *et al.* 1994; Marzoli *et al.* 2000). The isolation and the geographic characteristics promoted the divergence and speciation of the species, giving origin to high rates of endemism (Dutton 1994; Jones 1994; Drewes 2002; Measey *et al.* 2007; Uyeda *et al.* 2007; Vaz & Oliveira 2007; Melo *et al.* 2011). Regarding herpetofauna, with the exceptions of *Trachylepis affinis* in Príncipe, *Pelusios castaneus* (Schweigger, 1812) in São Tomé, and *Hemidactylus mabouia* (Moreau de Jonnès, 1818) and *Hemidactylus longicephalus* Bocage, 1873 on both islands (Manaças

1958), all species of reptiles appear to be endemic (Ceríaco *et al.* in press), while for the amphibians the rate of endemism is 100% (Ceríaco *et al.* in press). These islands have been the subject of many recent studies (e.g. Drewes & Stoelting 2004; Jesus 2005; Jesus *et al.* 2005a,b, 2006; Jones & Tye 2006; Measey *et al.* 2007, Jesus *et al.* 2007, 2009; Dallimer *et al.* 2009; Melo *et al.* 2011; Valle *et al.* 2014; Lima *et al.* 2015, 2016) leading to the identification and description of several new vertebrate species (e.g. Measey *et al.* 2007; Uyeda *et al.* 2007; Miller *et al.* 2012; Ceríaco 2015; Ceríaco *et al.* 2015; Bell 2016; Ceríaco *et al.* 2016, 2017).

The phylogenetic and phylogeographic relationships of the Gulf of Guinea islands *Trachylepis* were analyzed first at molecular level by Jesus *et al.* (2005a,b) and more recently at molecular and morphological level by Ceríaco *et al.* (2016). *T. maculilabris* (Gray, 1845) and *T. affinis* species include several highly divergent evolutionary lineages belonging to intricate complexes (Mausfeld *et al.* 2004; Rocha *et al.* 2010). Jesus *et al.* (2005a) showed that *T. affinis* had a high degree of differentiation (15%) between the Príncipe and Guinea-Bissau populations. The same study revealed that the two populations of *T. maculilabris* from São Tomé and Príncipe diverge 10.8% based on the cytochrome *b* and both have different lineages of unknown continental lineage (Jesus *et al.* 2005a). In a sequential study, Jesus *et al.* (2005b) obtained little or almost no gene flow between the two populations, showing a degree of geographic population substructuring within *T. maculilabris* consistent with the known geology of the island. This study contradicts the previous one (Jesus *et al.* 2005a) assuming that the populations of São Tomé and Príncipe are sister taxa and it assumes that the origin of all *T. maculilabris* lineages in São Tomé were about 800,000 ya. Later, Rocha *et al.* (2010) through molecular data again distinguished the populations of the two islands, and found that they do not form a monophyletic group. Recently, Ceríaco *et al.* (2016) through molecular and morphological data showed that the populations of São Tomé (*T. thomensis*) and Príncipe (*T. principensis*) are different from each other and from the continental population of *T. maculilabris*. It is possible that *T. thomensis* and *T. principensis* have the same ancestor and it is probable that *Trachylepis* colonized the two islands separately and not by island-hopping (Jesus *et al.* 2005a). Although there are few studies of *T. ozorii*, Jesus *et al.* (2005a) showed a high level of divergence (10.8% for cytochrome *b*) between *T. ozorii* and the other species of the genus, suggesting that this species is unrelated to the other *Trachylepis* species on the Gulf of

Guinea oceanic islands, proposing that the ancestors colonized Annobon directly from the continent, rather than "island-hopping". Despite the lack of available molecular data for *T. adamastor*, Ceríaco (2015) hypothesizes that this belongs to the same monophyletic lineage of *T. maculilabris* due to several synapomorphic characters. Either one of São Tomé or Príncipe populations may have given origin to the species of Tinhosa Grande, through the "island-hopping " colonization, a pattern known in the area by other reptile (Jesus *et al.* 2007) and amphibian species (Drewes & Wilkinson 2004). Considering this, there are still questions regarding the phylogeographic origin that need to be answered, since the hypothesis put forward by Ceríaco (2015) was formed only based on morphological data. To test this hypothesis, in February 2016 two field trips to Tinhosa Grande were held by the author, with the objective of investigating the presence of *T. adamastor* in the islet, knowing its habitat and collecting specimens and tissues. This study will focus on this question using mitochondrial, 16S (e.g. Mausfeld *et al.* 2000; Jesus *et al.* 2005a,b, 2007; Rocha *et al.* 2010; Ceríaco *et al.* 2016; Allen 2015; Allen *et al.* 2017) and ND2 (e.g. Portik *et al.* 2010, 2011; Portik & Bauer 2012; Allen 2015; Allen *et al.* 2017), and nuclear, RAG-1 (e.g. Portik *et al.* 2010, 2011; Portik & Bauer 2012; Allen 2015) genes, which are common in studies of this type for this genus.

Material and methods

Sampling

Fourteen individuals of *T. adamastor* were collected from the Tinhosa Grande islet, in 16 and 19 February 2017. For morphological and morphometric comparisons were also collected four *T. principensis* from Príncipe. Capture and handling of specimens were carried out with permission of the Regional Government of Príncipe and the direction of Obô National Park. The specimens were euthanized and preserved in 10% buffered formalin in the field and transferred to 70% ethanol after expedition. Liver tissue was removed before formalin fixation and preserved in 95% ethanol. The specimens collected were appropriately identified with labels for each one with a field number, associated with information such as coordinates, name of the collection site, collector (s), and date. The eighteen individuals collected were deposited in the Herpetological Collection of Museu Nacional de História Natural e da Ciência

(MUHNAC) (Lisbon, Portugal), with a new collection number, and their tissue samples were deposited in the Tissue and DNA Collection of MUHNAC. For mensural, merisitic, but also molecular comparisons were also included in the analysis the available data on *T. thomensis* (13 specimens), *T. principensis* (13 specimens) and *T. adamastor* (8 specimens), available on the collections of MUHNAC, California Academy of Sciences (CAS) (San Francisco, USA), Muséum d'Histoire Naturelle Genève (MHNG), (Switzerland), and Instituto de Investigação Científica Tropical (IICT) (Lisbon, Portugal) used in former papers (Ceríaco 2015; Ceríaco *et al.* 2016).

Morphological methods

The morphological analysis of the fourteen individuals of *T. adamastor* was performed, with three males, six females and five sub adults. For comparison, data from Ceríaco *et al.* (2016) were used, where specimens of *T. thomensis* and *T. principensis* (thirteen each) were examined; and also the data from Ceríaco (2015) for the *T. adamastor* (eight specimens). Specimens were measured only by the author with a digital caliper, lepidosis was observed with the help of binocular magnifiers. Scale nomenclature, scales counts and measurements used in the description follow Broadley (2000). The following characters were measured: snout-vent length (SVL), from the snout to the vent; tail length (TL), from cloaca to tip of tail, measured only in specimens with complete original tails; head width (HW); head length (HL), from tip of snout to anterior tympanum border; head height (HH), from the base of the maxilla to the top of head; eye-snout distance (ES), from the front of the eye to the tip of the snout; eye-nostril distance (EN), from the front of the eye to the nostril; inter-nostril distance (IN), minimum distance between the nostrils; number of scale rows at midbody (MSR); number of scales dorsally (SAD), from the nuchal (excluded from count) to base of the tail; number of scales ventrally (SAV), from the mental (excluded from count) to the anal plate (excluded); number of subdigital lamellae under Finger-IV (LUFF); number of subdigital lamellae under Toe-IV (LUFT); number of supralabials (SL), with those widened in subocular position indicated between brackets; number of supraciliaries (SC); number of supraoculars (SO); number of nuchal scales (NS); number of keels on dorsal scales (KDS); kind of contact between parietals (CP); kind of contact between frontoparietals (CFP); kind of contact between supranasals (CSN); kind of contact between prefrontals (CPF).

Molecular methods

During field work the liver tissue was removed from the specimens and preserved in the 1.5ml alcohol tube. This tube was identified with the field number of the specimen. The tissue was thus used to extract mitochondrial DNA according to protocol E.Z.N.A. ® Tissue DNA Kit Protocol - Tissue, in the Zoology Corridor genetics laboratory at MUHNAC. Two mitochondrial markers (mtDNA) were used, the 16S ribosomal unit and the NADH dehydrogenase subunit 2 (ND2), and one nuclear marker the RAG-1 (Table 1), for having been proven to be useful for identifying species level divergences in the genus *Trachylepis* (Mausfeld *et al.* 2000; Jesus *et al.* 2005a; Portik *et al.* 2010; Rocha *et al.* 2010; Ceríaco *et al.* 2016; Allen *et al.* 2017).

TABLE 1. Primers used in this study for the amplification of 16S, ND2 and RAG-1. Number of cycles, annealing temperatures and references of original primer publication.

Gene	Primer	Primer Sequences (5'–3')	cycles/ temp (°C)	Reference
16S	16SAR-L	CGCCTGTTTATCAAAAACAT	35/52	Palumbi 1996
	16SBR-H	CCGGTCTGAACTCAGATCACGT	35/20	Palumbi 1996
ND2	Metf1	AAGCTTTCGGGCCCATACC	34/50	Macey *et al.* 1997
	L5002	AACCAAACCCAACTACGAAAAAT	Sequencing primer	Macey *et al.* 1997
	CO1R1	AGRGTGCCAATGTCTTTGTGRTT	34/50	Arèvalo *et al.* 1994
	CO1R8	GCTATGTCTGGGGCTCCAATTAT	32/52	Weisrock *et al.* 2001
RAG-1	Rag1SkinkF2	TTCAAAGTGAGATCGCTTGAAA	34/50	Portik *et al.* 2010
	Rag1SkinkR1200	CCCTTCTTCTTTCTCAGCAAAA	34/50	Portik *et al.* 2010

Polymerase chain reaction (PCR) for 16S (PCT; 16 µl) consisted of 1x PCR Buffer 80 µl, 40 µl MgCl$_2$, 32 µl dNTP, 192.8 µl of H$_2$O, 8 µl primer16SAR-L and 8 µl 16SBR-H, 4 µl BSA (bovine albumin), 3.2 µl of Taq polymerase, and 1 µl of extracted DNA. Thermal cycling was as follows: initial denaturation step: 5 min at 85 °C; 35 cycles: denaturation 35 s at 94 °C, primer annealing for 3 s at 52 °C; extension for 1 min at 72 °C; and a final step of 5 min at 72 °C (Table 1). PCR reactions for ND2 and RAG-1 were performed with a final volume of 22.5 µl containing: 9.9 µl sterile H$_2$O, 0.1 µl of Taq DNA polymerase (New England BioLabs, Ipswich, MA, USA), and 2.5 µl each of

forward primer (8 ppm), reverse primer (8 ppm), 10x standard buffer (New England BioLabs), 5X-Q solution (Qiagen, Germantown, MD, USA), and dNTP mix. All PCR reactions were carried with an initial 2 min denaturation at 95˚C, followed by 32–34 cycles [35 s DNA denaturation at 95˚C, 35 s primer annealing at 50–52˚C, 1 min 35 s extension at 72˚C (Table 1)], and a final extension at 5˚C for 1 min. PCR products were tested for successful amplification using gel electrophoresis on 1.5% agarose gels. PCR products were cleaned using the protocol enonuclease I/FastAP™ Thermosensitive Alkaline Phosphatase. The samples were sent to a Macrogen company (Amsterdam, The Netherlands) for the sequencing. The obtained DNA sequences were individually aligned using the Squencher 4.8 software (Gene Codes, Ann Arbor, MI, USA) and manually analyzed for stop codons due to sequencing errors.

Phylogenetic analysis

Sequences were aligned using the ClustalW alignment tool in MEGA 6.06 (Tamura *et al.* 2013). A Maximum Likelihood (ML) analysis was performed on the 16S, ND2 and RAG1 dataset, using MEGA 6.06 (Tamura *et al.* 2013). Optimum substitution models were selected by ModelTest 3.7 (Posada & Crandall 1998) using the Akaike informative criterion. The analyses were performed with a random starting tree under the K2 + G model. Phylogenetic robustness was estimated in the ML analyses by running 1000 random addition bootstrap replicates (Felsenstein 1985). This study regarded tree topologies with bootstrap values (bs) of 70% or greater as well supported (Huelsenbeck & Hillis 1993). Pairwise comparisons of uncorrected sequence divergences (p-distance) were computed in MEGA 6.06 (Tamura *et al.* 2013). The specimens and sequences used for the genetic analysis and also their location are presented in the Appendix I.

Results

Morphology

Mensural and meristic data of the fourteen specimens *T. adamastor* are presented in Table 2. The general comparison between *T. adamastor* from Tinhosa Grande islet, *T. principensis* from Príncipe island and *T. thomensis* from São Tomé island are present in Table 3. The data of *T. adamastor* includes the specimens collected

on the expedition and also specimens already present at IICT collection from Ceríaco (2015). The data of *T. principensis* and *T. thomensis* was taken from Ceríaco (2015) and Ceríaco *et al.* (2016). The three species differ from each other, *T. adamastor* appears to have a much larger average of body size compared to the other two species (Maximum SVL *T. adamastor*: 112,04 mm, *T. principensis*: 88,30 mm, *T. thomensis*: 92,10 mm). Other characters such as head width (Maximum HW *T. adamastor:* 15,85 mm, *T. principensis:* 13,10 mm, *T. thomensis:* 13,5 mm), head length (Maximum HL *T. adamastor:* 22,37 mm, *T. principensis:* 19,90 mm, *T. thomensis:* 21,00 mm), head height (Maximum HH *T. adamastor:* 13,00 mm, *T. principensis:* 9,30 mm, *T. thomensis:* 11,10 mm), inter-nostril distance (Maximum IN *T. adamastor:* 4,18 mm, *T. principensis:* 2,80 mm, *T. thomensis:* 3,70 mm), eye-nostril distance (Maximum EN *T. adamastor:* 7,42 mm, *T. principensis:* 6,10 mm, *T. thomensis:* 6,20 mm), eye-snout distance (Maximum ES *T. adamastor:* 10,08 mm, *T. principensis:* 7,60 mm, *T. thomensis:* 8,80 mm) also have a higher average in *T. adamastor* specimens. *T. adamastor* (Fig. 1) is a more robust species when compared to the other two. Its coloration is also quite different from *T. principensis* (Fig. 2) and *T. thomensis* (Fig. 3), presenting a melanic coloration.

FIGURA 1. *Trachylepis adamastor* in life from Tinhosa Grande islet (Photo: Luis Ceríaco).

FIGURA 2. *Trachylepis principensis* in life from Príncipe island (Photo: Luis Ceríaco).

FIGURA 3. *Trachylepis thomensis* in life from São Tomé island (Photo: Luis Ceríaco).

TABLE 2. Measurements and scale counts of *Trachylepis adamastor*. Abbreviations are the same as those described in Materials and Methods.

	MB03-001015	MB03-001016	MB03-001017	MB03-001018	MB03-001019	MB03-001020	MB03-001043	MB03-001044	MB03-001045	MB03-001046	MB03-001047	MB03-001048	MB03-001049	MB03-001050
Sex	♀	♀	♀	♂	♀	♀	Subadult	Subadult	Subadult	Subadult	Subadult	♂	♀	♂
SVL	72,00	110,00	104,00	102,00	104,00	105,00	103,00	107,00	108,00	107,00	105,00	107,00	104,00	108,00
TL	76,00	132,00	145,00	123,00	134,00	121,00	99,00	115,00	23,00	95,00	129,00	96,00	65,00	95,00
HW	9,49	14,57	12,86	14,88	13,19	13,29	14,76	14,15	13,64	15,85	13,65	15,00	12,72	13,48
HL	12,15	17,05	17,22	18,59	17,04	17,80	18,08	17,12	16,93	18,20	17,17	17,99	17,48	18,82
HH	5,44	10,62	8,59	9,32	9,68	8,89	10,92	10,57	11,93	11,52	10,12	10,01	9,37	10,51
IN	2,82	3,34	3,82	3,78	3,52	3,39	4,08	3,97	4,06	4,18	3,61	4,03	3,78	3,75
EN	4,34	6,68	6,51	7,27	6,60	6,60	6,70	6,31	6,54	7,42	6,08	6,98	7,11	7,21
ES	6,71	9,49	9,02	9,78	9,09	10,02	10,08	9,00	9,76	9,78	9,63	9,44	9,04	10,07
HL/SV L (%)	16,88	15,50	16,56	18,23	16,38	16,95	17,55	16,00	15,68	17,01	16,35	16,81	16,81	17,43
ES/HL (%)	55,23	55,66	52,38	52,61	53,35	56,29	55,75	52,57	57,65	53,74	56,09	52,47	51,72	53,51
HH/HL (%)	44,77	62,29	49,88	50,13	56,81	49,94	60,40	61,74	70,47	63,30	58,94	55,64	53,60	55,84
HW/HL (%)	78,11	85,45	74,68	80,04	77,41	74,66	81,64	82,65	80,57	87,09	79,50	83,38	72,77	71,63
TL/SVL (%)	105,56	120,00	139,42	120,59	128,85	115,24	96,12	107,48	21,30	88,79	122,86	89,72	62,50	87,96
LUFT	13	14	14	14	13	13	13	14	14	13	14	13	13	14
LUFF	17	16	17	17	15	15	15	16	17	15	15	16	17	17
MSR	33	34	34	34	33	34	34	33	34	34	35	35	34	34
SAD	48	51	48	51	49	50	48	50	51	50	50	50	48	48
SAV	56	60	58	60	60	59	58	58	58	58	60	59	59	58
KDS	3	3-6	3-5	3-6	3-6	3-6	3-5	3	3-6	3	3-5	5-6	3-5	3-5

TABLE 3. General comparison between *Trachylepis* species from Tinhosa Grande islet, Príncipe and São Tomé islands. Data present as "min-max (mean ± standard deviation)". Abbreviations are the same as those described in Material and Methods.

	T. adamastor (n=22)	*T. principensis* (n=13)	*T. thomensis* (n=13)
Geographic distribution	Tinhosa Grande islet	Príncipe island	São Tomé island
SVL (mm)	72,00-112,04 (103,96 ± 10,03)	69,50-88,30 (81,75 ± 5,55)	74,00-92,10 (81,88 ± 6,54)
TL	23,00-175,64 (114,56 ± 10,03)	140,00-184,10 (157,06 ± 15,34)	116,3-180,00 (137,65 ± 17,14)
HW	9,49-15,85 (13,45 ± 1,74)	8,80-13,10 (10,87 ± 1,13)	9,00-13,5 (11,18 ± 1,25)
HL	12,15-22,37 (18,09 ± 2,36)	14,50-19,90 (17,56 ± 1,36)	15,30-21,00 (17,46 ± 1,58)
HH	5,44-13,00 (9,79 ± 1,84)	6,60-9,30 (8,12 ± 0,75)	6,30-11,10 (8,77 ± 1,48)
IN	2,50-4,18 (3,52 ± 0,49)	1,90-2,80 (2,38 ± 0,26)	1,50-3,70 (2,69 ± 0,60)
EN	4,34-7,42 (6,39 ± 0,82)	3,30-6,10 (4,87 ± 0,67)	3,90-6,20 (4,92 ± 0,80)
ES	6,71-10,08 (8,91 ± 1,06)	6,00-7,60 (6,82 ± 0,55)	6,10-8,80 (7,02 ± 0,85)
HL/SVL (%)	15,50-20,63 (17,40 ± 1,39)	20,10-24,10 (21,50 ± 1,44)	19,20-24,20 (21,35 ± 1,45)
ES/HL (%)	39,24-57,65 (49,65 ± 6,56)	36,10-41,60 (38,83 ± 1,88)	37,10-49,30 (40,22 ± 3,09)
HH/HL (%)	43,87-70,47 (54,10 ± 7,62)	41,80-81,40 (57,69 ± 16,18)	41,30-62,00 (49,91 ± 5,32)
HW/HL (%)	58,15-87,09 (74,64 ± 8,27)	55,30-65,90 (61,72 ± 2,75)	56,60-78,90 (63,91 ± 5,24)
TL/SVL (%)	21,30-164,95 (111,19 ± 39,42)	167,10-228,10 (198,44 ± 22,73)	145,20-205,10 (167,10 ± 21,27)
LUFT	13-20	20-23	15-20
LUFF	15-17	16-18	15-22
MSR	31-35	31-33	31-35
SAD	48-56	47-51	55-59
SAV	56-66	57-64	58-64
KDS	3-6	5-6	4-6
CP	Always in contact, or in contact in a single point	Always in contact, or in contact in a single point	Usually in contact, or in contact in a single point
CFP	Always in contact	Always in contact	Always in contact
CPF	Variable	Variable	Variable
CSN	Always in contact forming a suture	Always in contact	Always in contact
Coloration	Background color of the flanks and the upper side of head, neck, back, legs and tail are dark-brown, with many subtle white speckles on the dorsum starting on the neck and running through the entire dorsum to the base of tail. Subtle darker lateral band, starting on the extremities of the nuchals continues until the arm insertion. Lateral area of the body greyish, with some brown stains. Venter uniformly whitish. Supralabials with a whithish area on the base, black on the top.	Back uniformly brownish and belly light bluish on alcohol preserved specimens, and bluish to greenish in life specimens.	Back brownish, with some dark and white speckles and belly light orange-yellow in alcohol preserved specimens, pinkish-yellow in live specimens. A thin horizontal line composed by approximately seven to eight white speckles from the back of the eye to the top of the tympanum.

Phylogeny

The aligned dataset contains 3248 (572 16S; 1488 ND2; 1188 RAG1) molecular characters. Contrary to what was expected, at molecular levels *T. adamastor* does not differ from the Príncipe population, appearing to be conspecific. For the phylogenetic analysis of the different *Trachylepis* species using the mitochondrial marker 16S the following results were obtained. *T. thomensis* have a genetic distance of 2.6% from *T. principensis,* 3.5% from *T. affinis,* and 2.72% from *T. maculilabris. T. principensis* have a genetic distance of 4.17% from *T. affinis* and 2.56% from *T. maculilabris. T. affinis* is separated from *T. maculilabris* by a genetic distance of 3.97%. *T. adamastor* have a genetic distance of 2.6% from *T. thomensis,* 4.17% from *T. affinis*, 2.58% from *T. maculilabris. T. adamastor* have a genetic distance of only 0.02% from *T. principensis.* Using the mitochondrial marker ND2 the results were as follows: *T. thomensis* have a genetic distance of 3.2% from *T. principensis,* 3.7% from *T. affinis,* and 2.8% from *T. maculilabris. T. principensis* have a genetic distance of 3.69% from *T. affinis* and 2.9% from *T. maculilabris. T. affinis* is separated from *T. maculilabris* by a genetic distance of 4.13%. *T. adamastor* have a genetic distance of 3.2% from *T. thomensis,* 3.67% from *T. affinis*, 2.89% from *T. maculilabris. T. adamastor* have a genetic distance of only 0.3% from *T. principensis.* The results for the nuclear marker RAG-1 were: *T. thomensis* have a genetic distance of 1.2% from *T. principensis,* 2.96% from *T. affinis,* and 0.8% from *T. maculilabris. T. principensis* have a genetic distance of 3.2% from *T. affinis* and 1% from *T. maculilabris. T. affinis* is separated from *T. maculilabris* by a genetic distance of 2.65%. *T. adamastor* have a genetic distance of 1.15% from *T. thomensis,* 3.28% from *T. affinis*, 1.1% from *T. maculilabris. T. adamastor* have a genetic distance of only 0.4% from *T. principensis.* All values, for both mitochondrial and nuclear markers, are greater than the maximum divergence found within the same species, except for *T. adamastor* and *T. principensis. Trachylepis affinis* was used as outgroup because it was clear its separation and distance from the group under analysis. Phylogenetic analysis for 16S mitochondrial gene shows that the monophyletic group of *T. maculilabris* complex contains within itself a considerable cryptic diversity (Fig. 4). Considering that *T. maculilabris* was originally described from West Africa (Gray 1845), it is assumed that this lineage represents the topotypic population. Outside the topotypic population are two other *T.* cf. *maculilabris, T.* cf. *maculilabris* 1 from Socotra Archipelago, Yemen (JQ598785-1) and *T.* cf. *maculilabris* 2 from Tanzania

(AY070356.1). The presence of these second lineage and potential cryptic species had already been reported by other authors (Mausfeld *et al.* 2000; Mausfeld *et al.* 2004; Rocha *et al.* 2010). Besides this second lineage, two other lineages are of interest and potentially represent species candidates: *T.* cf. *maculilabris* 3 from Burundi (CAS250828) and *T.* cf. *maculilabris* 4 from Gabon (CAS258254). The lineages of the São Tomé and Príncipe and its islets populations fall within the expanded *T. maculilabris* complex. These results confirm those of Ceríaco *et al.* (2016), which show two different species for the two islands, *T. thomensis* (São Tomé) and *T. principensis* (Príncipe). However, although the results for *T. adamastor* confirm the hypothesis presented by Ceríaco (2015), in which the author noted that *T. adamastor* would belong to the *T. maculilabris* species complex and with close affinities to either *T. principensis* or *T. thomensis*, the tree shows that *T. adamastor* and *T. principensis* belong to the same branch, presenting only a very small structure that reflects the isolation of the populations but does not allow to affirm that they are different species. Although for the mitochondrial ND2 gene some of the *T. maculilabris* populations are not available, the topology of the tree (Fig. 5) reflects the same relationships as the 16S gene. The *T. principensis* and *T. adamastor* appear conspecific, sister of *T. maculilabris* West Africa, while *T. thomensis* appears as sister of these two. The nuclear gene RAG-1, despite lacking as much taxonomic and population coverage as the latter genes still presents the same topology (Fig. 6) as that of the mitochondrial genes.
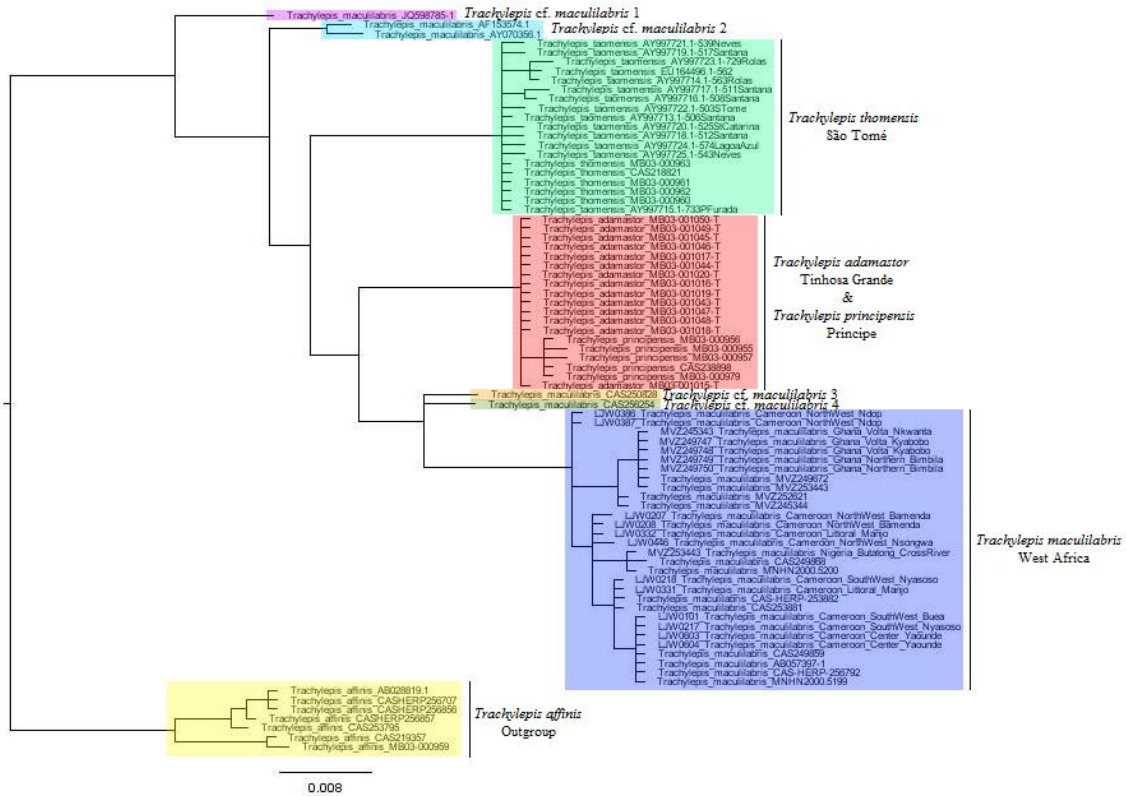
FIGURE 4. Maximum-likelihood phylogeny of 16S gene dataset of *Trachylepis*.
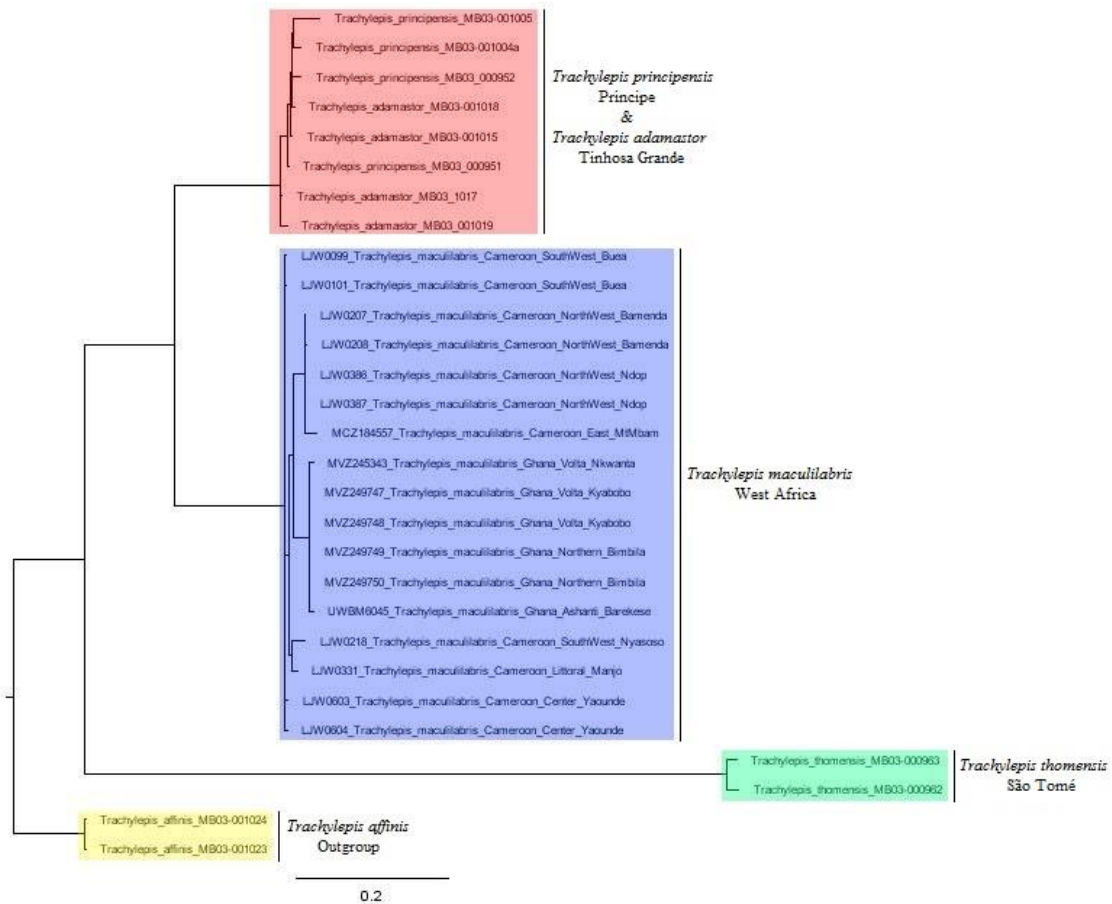
FIGURE 5. Maximum-likelihood phylogeny of ND2 gene dataset of *Trachylepis*.
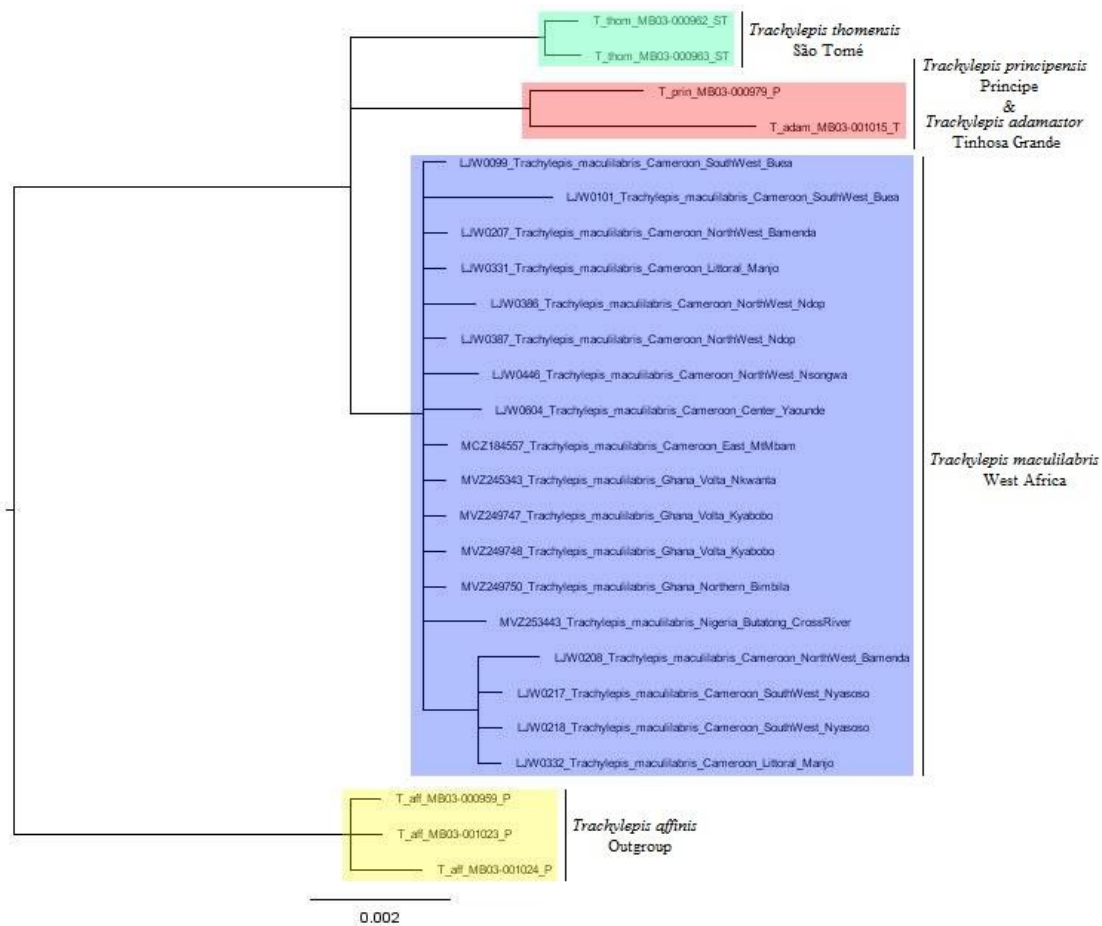
FIGURE 6. Maximum-likelihood phylogeny of RAG-1 gene dataset of *Trachylepis*.

Discussion

Based on the two mitochondrial (16S and ND2) and one nuclear (RAG-1) genes, *T. adamastor* and *T. principensis* belong to the same clade. These results partly agree with the initial findings of Ceríaco (2015) and Ceríaco *et al.* (2016) regarding the relationship between the São Tomé and Príncipe species, but contradicts the hypothesis that *T. adamastor* represents a separate species from the Príncipe species. However, both Ceríaco (2015) and Ceríaco *et al.* (2016) and this study found consistent morphological differences between the three populations. Morphologically *T. adamastor* is very different from *T. principensis*, with a more robust body and with a melanic coloration. However molecular data show that the population of Príncipe island (*T. principensis*) and Tinhosa Grande islet (*T. adamastor*) are conspecific, and share the same ancestral of the sister species *T. thomensis* and *T. maculilabris.* The colonization of *T. principensis* and *T. thomensis* probably occurred separately and not by island-hopping, and possibly these two populations have a common ancestor (Jesus *et al.* 2005a).

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Two similar cases to that of *adamastor/principensis* are known in the *Gallotia* Boulenger, 1916 and *Podarcis* Wagler, 1830 genera. The species *Gallotia simonyi* (Steindachner, 1889) endemic from Canary island, which was for a long period of time considered to be extinct from the small island Roque Chico of Salmor by the scientific community (Carranza *et al.* 1999), contains two subspecies which were described based on morphological differences - the extinct subspecies *Gallotia simonyi simonyi* (Steindachner 1889) of the small island Roque Chico of Salmor and the subspecies *G. s. machadoi* López-Jurado 1989 of the island El Hierro. These differences were related to the size, robustness, shape of the pileus, head and number of temporal scales (Machado 1985; López-Jurado 1989). Despite these morphological differences Carranza *et al.* (1999) found no genetic differentiation between both subspecies. The other case is that of *Podarcis bocagei berlengensis* Vicente, 1985 from the Berlengas islands off the western coast of Portugal, which was initially described by Vicente (1985) as a new subspecies, due to its relatively larger form and coloration patters. However, this subspecies was also found near the adjacent coast in Peniche (Sá-Sousa 1992 *in* Sá-Sousa *et al.* 2000). Later, Harris & Sá-Sousa (2001) using the CO1 mitochondrial gene did not find genetic differences between *Podarcis b. berlengensis* and *P. carbonelli* Pérez Mellado, 1981 (from the mainland), then changed the name to *P. c. berlengensis* (see also Sindaco & Jeremčenko 2008). During the last glacial maximum (LGM) (occurring between 19,000 to 21,000 years ago (Mix *et al.* 2001; Clark *et al.* 2009)) Príncipe island extended considerably to south, covering the region where today Tinhosas islets are, and only became separated again after the rise of the sea levels following the LGM. Thus, it is possible that the Tinhosa population (*T. adamastor*) represent an isolated population from the once larger Principe island that underwent a rapid adaptation, which was much faster phenotypically than genetically. With the passing of the years of separation between the Príncipe and the Tinhosas, the Tinhosa Grande islet suffered erosion and was devoid of any vegetation. *T. adamastor* had to adapt to this inhospitable habitat, which its giant form may be due to the relaxation of predation (Case 1978, 1982; Lomolino 1985; Grant 1998), such as the absence of mammal predators (Szarski 1962; Case 1978, 1982; Pregill 1986; Greer 2001; Meiri 2008) or due to the presence of ten thousand seabirds in the island (Sanchez-Piñero & Polis 2000; Bonnet *et al.* 2002), the accumulation of fecal matter, food remains and carcasses increase the availability of nutrients (Polis & Hurd 1996), or both. The melanic coloration of *T. adamastor* can be a protection against ultraviolet (UV)

radiation (Kollias *et al.* 1991; Setlow *et al.* 1993; Gunn 1998; Hofer & Mokri 2000; Callaghan *et al.* 2004; Calbó *et al.* 2005), since it is very exposed to sunlight, a resistance against diseases (Wilson *et al.* 2001), or an immune function (Mackintosh 2001; Wilson *et al.* 2001), among others. The origin of new morphologies and species are closely related to adaptive radiations, rapid divergence and phenotypic diversification (Simpson 1953; Givnish & Systma 1997; Schluter 2000; Glor 2010; Losos & Mahler 2010), and the present case falls under the main conditions of any classical case of adaptive radiation, that includes the Darwin's finches on the Galápagos islands (Grant & Grant 2002; Grant *et al.* 2004; Petren *et al.* 2005), *Anolis* lizards on Caribbean islands (Losos 1992; Irschick *et al.* 1997; Pinto *et al.* 2008), cichlids of the East African Great Lakes (Sturmbauer 1998; Seehausen 2006) and Hawaiian silverswords (Baldwin 1997; Barrier *et al.* 1999), among others (Givnish & Systma 1997; Schluter 2000).

The results of phylogenetic relationships between Príncipe and Tinhosa Grande populations should be considered, despite genetically conspecific, they are in fact very different from each other morphologically. Furthermore, it is important to see this case in the light of species concept in similar cases, since these concepts are still currently surrounded by considerable controversy, leading to the fact that there are no fixed or established limits to define what it is or what is not a species. Besides this, many authors consider the subspecies concept among species, an even more debatable concept and without consensus among the scientific community.

For this particular case, there are two possible solutions. The first one, would be to consider both populations conspecific. According to the Code (ICZN 1999) article 23 (Principle of Priority), the valid name of the population from Príncipe and from Tinhosa Grande is the oldest available name, which in this case it would be *T. adamastor*, and therefore *T. principensis* would be sunk into *adamastor* synonymy. The second option, would be to consider each island population as a subspecies, *T. adamastor adamastor* for the population of Tinhosa Grande islet and *T. adamastor principensis* of the Príncipe island, given their striking morphological differences and its allopatry. This option was that followed by Sá-Sousa *et al.* (2000), Harris & Sá-Sousa (2001) and Sá-Sousa & Harris (2002) for the case of the Berlengas Wall lizard already mentioned above. The choice between any of these options bears consequences, since it will critically affect the way decisions are going to be made regarding their conservation. In the case of the

adoption of the second option, each population would be considered an operational taxonomic unit (OTU). A given OTU groups and classifies different species populations, groups or closely related individuals, and can also have different designation, as for example evolutionarily significant units (ESUs) (Ryder 1986; Moritz 1994), operational units (Diniz-Filho & Telles 2002) and management units (MUs) (Moritz 1994). More recently, for standard taxonomic and ecological surveys were proposed by Blaxter *et al.* (2005) the molecular operational taxonomic units (MOTUs). These classifications help to prioritize in several domains, in effective way, taxa that are related.

Considering the results of the phylogeny and the relationships of the three populations of the São Tomé and Príncipe islands and Tinhosa Grande islet it is suggested, until new data are available, that *T. adamastor* be subdivided into two subspecies, *T. adamastor adamastor* for Tinhosa Grande population and *T. adamastor principensis* for Príncipe population. This suggestion is due to the fact that *T. adamastor* has undergone an allopatric speciation, the two populations are geographically separated by the ocean, so this isolation makes their contact difficult. Furthermore, the Tinhosa Grande population clearly had a radical adaptation, which resulted in very pronounced morphological differences, suggesting that these two populations are diverging. The islet conditions and isolation make *T. adamastor adamastor* vulnerable when submitted to different pressures and more susceptible to external events as predation and competition of introduced animals, environmental issues as climate change or any other stochastic events. Considering these issues, the risk of local extinction is possibly high, and given its small distribution area, more investigation is need not only to deepen knowledge of this population, but also to considerer suitable conservation plans focused on this population in particular. Following the option of dividing the two populations into subspecies, *T. adamastor adamastor* remains one of the most threatened animals on the planet due to its limited distribution, and for this reason two conservation plans must be considered (on for *T. adamastor adamastor* and other for *T. adamastor principensis*). *T. adamastor adamastor* is in a very vulnerable situation, so the national authorities should take action as soon as possible to protect this unique and endemic subspecies.

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Appendix I - Specimens and sequences used for the genetic analysis.

Name on trees	Specimens and/or sequences	Localization
Trachylepis cf. *maculilabris* 1	JQ598785-1	Socotra Archipelago, Yemen
Trachylepis cf. *maculilabris* 2	AF153574.1	N/A
	AY070356.1	Tanzania
Trachylepis cf. *maculilabris* 3	CAS250828	Burundi
Trachylepis cf. *maculilabris* 4	CAS258254	Gabon
Trachylepis maculilabris	LJW0386	Cameroon
	LJW0387	
	LJW0207	
	LJW0208	
	LJW0332	
	LJW0446	
	LJW0218	
	LJW0331	
	LJW0101	
	LJW0217	
	LJW0603	
	LJW0604	
	LJW0099	
	MCZ184557	
	CAS249868	
	MNHN2000.5200	
	CAS-HERP-253882	
	CAS253881	
	CAS249859	
	AB057397-1	
	CAS-HERP-256792	
	MNHN2000.5199	
	MVZ245343	Ghana
	MVZ249747	
	MVZ249748	
	MVZ249749	
	MVZ249750	
	UWBM6045	
	MVZ249672	
	MVZ252621	
	MVZ245344	
	MVZ253443	Nigeria
	MVZ253443	
Trachylepis adamastor	MB03-001050-T	Tinhosa Grande
	MB03-001049-T	
	MB03-001045-T	
	MB03-001046-T	
	MB03-001017-T	
	MB03-001044-T	
	MB03-001020-T	
	MB03-001016-T	
	MB03-001019-T	
	MB03-001043-T	
	MB03-001047-T	
	MB03-001048-T	
	MB03-001018-T	
	MB03-001015-T	
Trachylepis principensis	MB03-000956	Príncipe
	MB03-000955	

	MB03-000957	
	CAS238898	
	MB03-000979	
	MB03-001005	
	MB03-001004A	
	MB03_000952	
	MB03_000951	
Trachylepis thomensis	AY997721.1-539	São Tomé
	AY997719.1-517	
	AY997723.1-729	
	EU 164496.1-562	
	AY997714.1-563	
	AY997717.1-511	
	AY997716.1-508	
	AY997722.1-503	
	AY997713.1-506	
	AY997720.1-525	
	AY997718.1-512	
	AY997724.1-574	
	AY997725.1-543	
	MB03-000963	
	CAS218821	
	MB03-000961	
	MB03-000962	
	MB03-000960	
	AY997715.1-733	
Trachylepis affinis	AB028819.1	Príncipe
	CASHERP256707	
	CASHERP256856	
	CASHERP256857	
	CAS253796	
	CAS219357	
	MB03-000959	
	MB03-001024	
	MB03-001023	

Paper II: Ecology and conservation status of an endemic Skink, *Trachylepis* (Squamata: Scincidae), from Tinhosa Grande islet, Gulf of Guinea.

Abstract

The population density of the Adamastor skink, *Trachylepis adamastor adamastor*, in Tinhosa islet is currently unknown. This is also true for the basic natural history and ecology of the subspecies. This study includes the first population density estimation and diet analysis of *T. a. adamastor*, in order to increase knowledge about the ecology of this species. The estimated population density of *T. a. adamastor* were 0.012 per m^2 and the estimated population size were 2460 individuals on Tinhosa Grande islet. Regarding these results the IUCN Red List criteria classifies *T. a. adamastor* as "Critically Endangered". The diet included 13 prey types, mostly Acari, Formicidae and Araneae were found. *T. a. principensis* and *T. thomensis* stomach contents were also analyzed for comparison.

Key words: *Trachylepis*, São Tomé & Príncipe, Tinhosa Grande, population density, diet, conservation.

Resumo

A densidade populacional da lagartixa Adamastor, *T. adamastor adamastor,* no ilhéu da Tinhosa é atualmente desconhecida. Assim como a sua história natural e ecologia. Este estudo inclui a primeira estimativa da densidade populacional e a análise da dieta de *T. a. adamastor*, a fim de aumentar o conhecimento sobre a ecologia desta espécie. A estimativa da densidade populacional de *T. a. adamastor* foi 0.012 por m^2 e a estimativa do tamanho da população foi de 2460 indivíduos no ilhéu da Tinhosa Grande. De acordo com estes resultados os critérios da Lista Vermelha da IUCN classificam *T. a. adamastor* como "Criticamente em Perigo". A dieta incluiu 13 tipos de presa e foram encontrados maioritariamente Acari, Formicidae e Araneae. Os conteúdos estomacais de *T. a. principensis* e *T. thomensis* também foram analisados para comparação.

Palavras chave: *Trachylepis*, São Tomé e Príncipe, Tinhosa Grande, densidade populacional, dieta, conservação.

Introduction

Wildlife management and conservation are one of the world's most important concerns today. The development of effective management and conservation plans and measures for a specific species requires knowledge on about population levels, natural history, geographic distribution and ecological habits (IUCN 2001).

The current International Union for Conservation of Nature (IUCN) criteria for defining conservation status are profoundly based on data about the populations of the evaluated taxa (IUCN 2001). Population densities are dependent of the availability of resources (relative importance of environmental) and the distribution of these resource between the species that share the same distribution range (ecological constraints) (Damuth 1981, 1987; Tilman 1994). These constraints are particularly important on islands, given their extinction and colonization dynamics, isolation, reduced species diversity and the relatively small number of interactions across species, all of which modify the environmental equilibrium and ecological control on population dynamics (MacArthur & Wilson 1967; Wright 1980). The success of survival and reproduction is directly related with morphology, physiology and with the capability to find and capture preys, this is also influenced by prey abundance, type of habitats, distribution, predators and competitors (Eifler & Eifler 1999).

Reptile populations are especially difficult to quantify due to their small body size, habitat preferences, secretive behavior and fast unpredictable activity (Turner 1977). Sampling methods for the study of herpetofauna are a very variable and depedant of the different taxa that need to be sampled (e.g. Campbell & Christman 1982; Mengak & Guynn 1987; Pearman *et al.* 1995; Rodda *et al.* 2001; Doan 2003; Anton *et al.* 2014), but the most widely used methods involve either sighting animals in line transects (Buckland *et al.* 1993; Cassey & Ussher 1999; Anderson *et al.* 2001), capture-mark-recapture techniques (Ballinger & Congdon 1981; Pollock *et al.* 1990; Cassey & Ussher 1999; Kwiantkowski & Sullivan 2002; Grant & Doherty 2007; Kacoliris *et al.* 2009; Efford & Fewster 2013), live-trapping (Mengak & Guynn 1987; Efford 2004), quadrats (Sumner *et al.* 2001; Doan 2003), distance-sampling (Buckland *et al.* 2001; Rodda & Campbell 2002; Grant & Doherty 2007; Kacoliris *et al.* 2009; Smolensky & Fitzgerald 2010), and visual encounter survey (Crump & Scott 1994; Doan 2003).

Lizards populations that inhabit small islands presents some differences when compared with their relative of the continent, which are attributed to the different selective pressures on islands, since insular lizards populations have generally a relatively limited habitat heterogeneity, food resources and low predation or interspecific competition pressures (Rocha *et al.* 2009). On small islands lizards tend have a larger body size (Case 1978, 1982; Russel & Bauer 1986; Greer 2001), a small clutch and litter size, large eggs and neonate size (Fitch 1985; Galán & Vincent 2003) and a greater plant consumption (Cooper & Vitt 2002; Olesen & Valido 2003).

Among lizards, the skinks (family Scincidae) are an extraordinarily speciose group with about 1,613 species (Uetz & Hošek 2016), they are the most diverse lizards in the world characterized by their variety of body characteristics – as color morphs, sizes, presence/absence of limbs, etc. Also, they are known for their high ecological diversity (mainly in the tropic regions), due to their wide distributions, which includes huge diversity of different biomes and habitats (Pianka & Vitt 2003).

Trachylepis adamastor adamastor Ceríaco, 2015, is an endemic skink from the Tinhosa Grande islet (area approximately 20.5 ha) located approximately 20 km southwest of the Príncipe island (Ceríaco 2015). The Tinhosas are known by their important nesting site for several seabirds in West Africa, include in the RAMSAR convention (RAMSAR 2017) and integrated in the Biosphere Reserve of Príncipe. *T. a. adamastor* shares the Tinhosa Grande islet with an unknown species of *Hemidactylus*, whose identity is currently being studied (Ceríaco *et al.* in prep.). Data on ecology and trophic relations, population density, and conservation status for *T. a. adamastor* is presently unavailable (Ceríaco 2015).

T. a. adamastor seems to have experienced adaptive radiation after the isolation of Tinhosa islet from Príncipe island (see the first paper of this thesis), which from the trophic point of view suggests that the species should present a generalist and opportunistic diet. The limited data presented by Ceríaco (2015), which noted that some individuals were observed feeding on split eggs of nesting birds (Ceríaco 2015), seems to corroborate this hypothesis. Rocha *et al.* (2004) in a diet study of an insular population of the skink *Brasiliscincus agilis* (Raddi, 1823) (family Scincidae), observed 11 types of prey, which the authors characterized as an example opportunistic predator. It is possible that the same can happen with *T. a. adamastor*.

There are several studies regarding the diet of Scincidae species worldwide (James 1991; Twigg *et al.* 1996; Craig *et al.* 2007; Rocha *et al.* 2004; Rocha *et al.* 2009), but only few are related African species (but see Akani *et al.* 2002; Akani *et al.* 2009; Eniang *et al.* 2014). Considering their diversity of habitats and the seasonal regimes in Africa regions, dietary variations are expected within the same species and possibly in the same geographic region (Eniang *et al.* 2014).

Conservation-wise, given the small and isolated area of the Tinhosa Grande and the insular nature of *T. a. adamastor*, an unexpected perturbation, as species introduction, may endanger the population, given the typical lack of response capacity to unpredicted events of insular populations (Barahona *et al.* 2000; Ceríaco 2015). Despite Tinhosa Grande islet being part of RAMSAR convention and being in the highlight for Bird Conservation plans, effective protection of the reserve is the most important measure to ensure the survival of *T. a. adamastor*. However new data about *T. a. adamastor*, density, ecology and threats are crucial for designing any conservation plan.

In this paper is present the first ecological and population size data for the species, as well as list potential conservation strategies based on these data.

Material and methods

Study area

The Tinhosas are two small rocky islets located to approximately 20 km to the southwest of Príncipe, the Tinhosa Grande (N: -1,34135556, E: -7,29151389; WGS84) has a surface area of about 20.5 ha and the Tinhosa Pequena (N: -1,38241667, E: -7,28316944, WGS-84) has only 3.3 ha (Leventis & Olmos 2009). The Príncipe island belongs to the Democratic Republic of São Tomé and Príncipe, located in the Gulf of Guinea, have never been connected to the mainland (Jones 1994; Lee *et al.* 1994). The Tinhosas are classified as important area for birds (IBA) (BirdLife International 2017) and are also recognized as Wetlands of International Importance and official Waterfowl Habitat (RAMSAR 2017). Tens of thousands of breeding pairs of seabirds (Leventis & Olmos 2009; Valle *et al.* 2014; BirdLife International 2017) use the islets to nest (Fig. 1) being one of the most important seabird colonies in West Africa (Jones & Tye 2006). Land crabs, termites, spiders and other arthropods also inhabit the islet (pers. obs.).

FIGURE 1. Tinhosa Grande islet with tens of thousands seabirds of breeding pairs (Photo: Luis Ceríaco).

Specimen collecting

Fourteen individuals *T. a. adamastor* were collected on Tinhosa Grande. For morphological, morphometric and diet comparisons were also collected four *T. a. principensis* from Príncipe. Capture and handling of specimens were carried out with permission of the Regional Government of Príncipe and the direction of Obô National Park. The specimens were euthanized and preserved in 10% buffered formalin in the field and transferred to 70% ethanol after expedition. Liver tissue was removed before formalin fixation and preserved in 95% ethanol. The eighteen individuals collected were deposited in the Herpetological Collection of Museu Nacional de História Natural e da Ciência (MUHNAC), Lisbon (Portugal), and their tissue samples were deposited in the Tissue and DNA Collection of MUHNAC. For diet comparisons were also examined *T. a. principensis* and *T. thomensis* specimens from the collections of the MUNHAC and the eight *T. a. adamastor* specimens from the collections of the Instituto de Investigação Científica Tropical, Lisbon, Portugal (IICT).

Fieldwork and Population estimates

Sampling was performed using the quadrat sampling method. Given the homogeneous habitat of the islet, individuals were counted in 20 quadrats of 10x10 m (adapted from Valle *et al.* 2014) each day, randomly selected along a transect line. Quadrats were distanced at random distances. The samples are represented in Fig. 2.
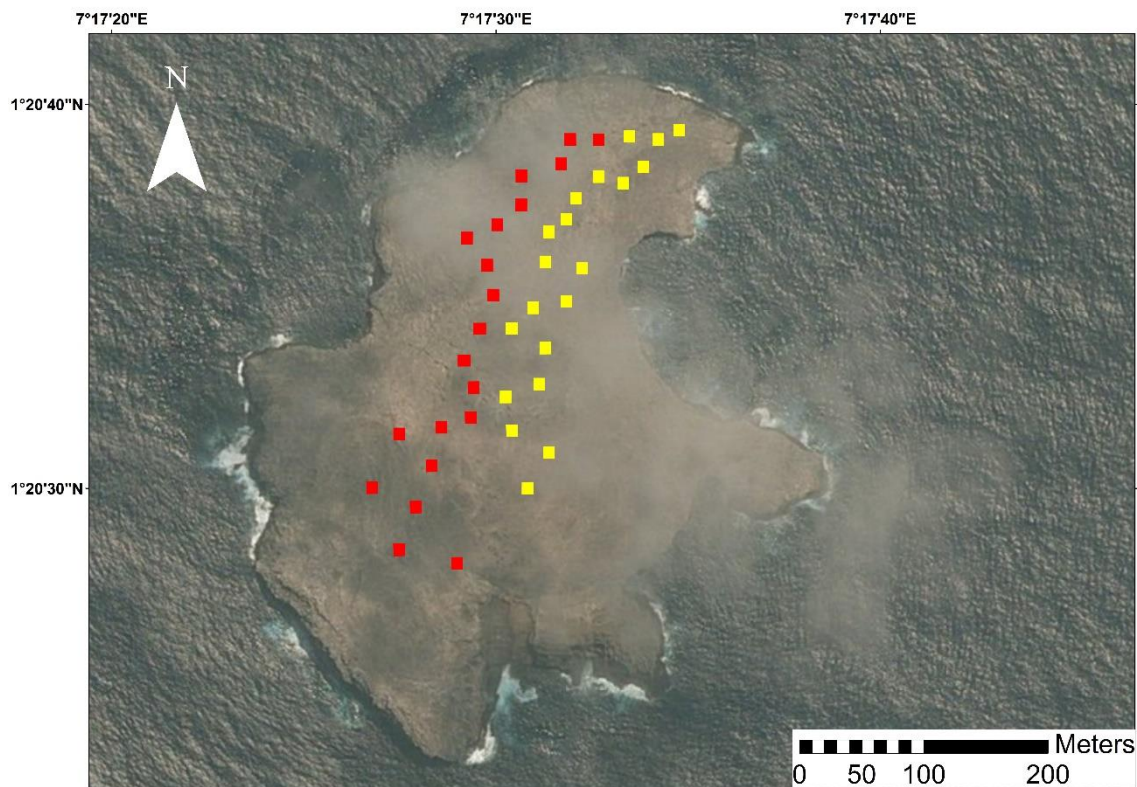
FIGURE 2. Quadrats transect in the Tinhosa Grande islet. Yellow quadrats represent the survey conducted on February 16th of 2016, while the red quadrants represent the second day of prospections on February 19th of 2016.

The sampled area (4000 m²) represents 1,95% of the total area (20.5 ha=205000.0 m²). The harsh conditions of the islet, with irregular, rocky and loose terrain and steep boundaries, the limited prospecting time (only two days), combine with the seabird nesting height (resulting in thousands of nests and new born birds), created constrains that had complicated the sampling process. The population estimate was calculated using The Ratio Method (adapted from Krebs 1989), although this method is being used in census with aerial transects, for large mammals in areas of difficult access. This was the most adequate method due to the available data, found in the bibliography.

To calculate average density for the whole area the following formula was used:

Average density $= \hat{D} = \dfrac{\text{Total individuals counted}}{\text{Total area searched}} = \dfrac{\sum Xi}{\sum Zi}$

where Xi = total individuals counted in quadrat i
   Zi = area of quadrats i
   i = sample number (1, 2, 3…n)
   n = total number of quadrats

And to estimate the total population the following:

$\hat{P} = \hat{D}A$

where   A = total area
   $\hat{D}$ = average density per unit area

Stomach contents/diet

After the morphological and morphometric analysis, the 14 specimens of *T. a. adamastor* and 4 specimens of *T. a. principensis* were dissected for stomach content analysis. After excision, the stomachs were opened, and their contents were preserved in an alcohol tube (70% ethanol) identified with the number of the respective specimen. Only 11 of the 14 *T. a. adamastor* had contents. The stomach content of 1 specimen of *T. a. principensis*, 9 specimens of *T. thomensis* (5 from Rolas Islet and 4 from São Tomé island), from the IICT collection (already reviewed by Manaças 1958) were also analysed and used for the comparison. For the analysis, the contents were spread on a Petri dish and examined under a microscope. The samples were grouped by *Trachylepis* species, and the prey items were identified to the lowest taxonomic level possible and were grouped accordingly.

Results

Population estimates

A total of 48 individuals *T. a. adamastor* were counted, with a total area searched of 4000 m^2 (Table 1).

TABLE 1. Number of *Trachylepis a. adamastor* records counted in the quadrats transect during the field trips in 16th and 19th February of 2016.

Date	16 February																				19 February																			
Site nº	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Number of *T. adamastor* specimens counted	2	3	1	2	0	2	0	1	0	3	0	0	1	0	2	0	4	1	0	1	2	2	1	0	3	0	2	1	1	1	2	1	0	3	3	1	0	0	2	0

In relation to the study site, the average density was 0.012 *T. a. adamastor* per m^2. Calculations using The Ratio Method (adapted from Krebs 1989) showed an estimated population size of 2460 individuals inhabitants in Tinhosa Grande islet.

Stomach contents/diet

The diet of *T. a. adamastor, T. a. principensis* and *T. thomensis* is presented in Table 2 (see also Appendix I). The majority of the prey items consisted of arthropods, with no plant material found. In addition to arthropods, egg shells, Acari seedlings, feathers, stones and undetermined arthropod remains (legs and wings), were also found. One hand of a *T. a. adamastor* was also found in the stomach contents of one individual (MB03-001017). Cumulatively, the most important prey items in the diet of the three *Trachylepis* species were Acari, Araneae, Coleoptera and Formicidae (Table 3). Although Acari have been the most numerous (145 items), only the *Trachylepis a. adamastor* presented it in his diet.

TABLE 2. Summary of dietary data, collected from stomach contents of the three *Trachylepis* species from Tinhosa Grande islet, Príncipe and São Tomé islands.

	Prey Type	*Trachylepis a. adamastor*	*Trachylepis a. principensis*	*Trachylepis thomensis*
Phylum	Arthropoda	1	-	-
Class	Chilopoda	4	1	-
	Symphyla	2	-	-
Subclass	Acari	145	-	-
Order	Araneae	10	6	4*
	Coleoptera	1	4	7*
	Coleoptera larvae	1	-	-
	Collembola larva	1	-	-
	Dermaptera	-	-	-
	Diptera	-	2	1
	Diptera larvae	-	-	15
	Hymenoptera	-	9	2
	Isopoda	-	1	-

	Lepidoptera larvae	1	-	-
	Mantodea	-	-	1
	Thysanoptera	-	-	-
	Thysanura	1	-	-
Suborder	Caelifera	-	1	-
	Homoptera	-	9	2
	Homoptera nymph	1	-	-
Family	Curculionidae	-	1	-
	Formicidae	15	9	4
	Tettigoniidae	-	1	-
	Total	**183**	**44**	**36**

*- undetermined number

TABLE 3. Number total of items prey found, frequency of occurrence and relative frequency for each prey item for *Trachylepis a. adamastor*, *Trachylepis a. principensis* and *Trachylepis thomensis* species.

	Items	Number	Frequency	Relative Frequency
Phylum	Arthropoda	1	1	1,49
Class	Chilopoda	5	3	4,48
	Symphyla	2	2	2,99
Subclass	Acari	145	10	14,93
Order	Araneae	20	10	14,93
	Coleoptera	12	8	11,94
	Coleoptera larvae	1	1	1,49
	Collembola larvae	1	1	1,49
	Dermaptera	3	1	1,49
	Diptera	3	3	4,48
	Diptera larvae	15	1	1,49
	Hymenoptera	11	3	4,48
	Isopoda	1	1	1,49
	Lepidoptera larvae	1	1	1,49
	Mantodea	1	1	1,49
	Thysanoptera	1	1	1,49
	Thysanura	1	1	1,49
Suborder	Caelifera	1	1	1,49
	Homoptera	11	3	4,48
	Homoptera nymph	1	1	1,49
Family	Curculionidae	1	1	1,49
	Formicidae	28	11	16,42
	Tettigoniidae	1	1	1,49
	Total	**267**	**67**	**100,00**

Discussion

The data collected during the survey allowed a first input to the knowledge of the possible approximation of the *T. a. adamastor* population density. The estimated density for *T. a. adamastor* were 0.012 per m^2 on the Tinhosa Grande islet. Comparing this case with other similar studies on islands (López-Victoria *et al.* 2013; Hughes *et al.* 2015; Williams *et al.* 2015), the average density of *T. a. adamastor* were very low (e.g. *P. transversalis* 0.12 per m^2, on Malpelo Island, López-Victoria *et al.* 2013; *P. pulcher* 0.04 per m^2, on Lesser Antillean island of Barbados, Williams *et al.* 2015; *P. martini* 0.02 per m^2, on Curaçao Island, Hughes *et al.* 2015). However only about 2% of the islet was sampled, during a very limited time period. These limitations were a result of the short time available, the difficult logistics and the climacteric conditions of the islet and sea. Although these values are insufficient to obtain a more robust result, which would surely be achieved through a more extensive sampling, the presented data is of considerable importance. If these results are representative, *T. a. adamastor* should be classified as "Critically Endangered" following IUCN Red List criteria (IUCN 2001), according to it extent of occurrence (B1 - extent of occurrence estimated to be less than 100 km^2, although it was not estimated, considering the total area of the islet, 0.2050000 km^2, the extent of occurrence of *T. adamastor* will be lower than 100 km^2) and area of occupancy (B2 - area of occupancy estimated to be less than 10 km^2, 0.2050000 km^2 total area of the Tinhosa Grande islet). Further surveys will be needed to corroborate these results.

The diet of the three *Trachylepis* species included a relatively wide range of prey items types (20 in total), which characterizes a generalist and opportunistic predator. The three species consumed basically arthropods with no ingestion plant material. Some studies with *Trachylepis* genus denote that predominantly insectivorous skinks have normally no or an irrelevant plant material in their diet (Huey & Pianka 1977; Castanzo & Bauer 1998; Wymann & Whiting 2002). The most abundant arthropods found, considering the total abundance in the three *Trachylepis* species studied, were Araneae (more than 20 items), Coleoptera (more than 13 items), Diptera (18 items), and Formicidae (28 items). The prey items include both relatively sedentary (e.g. larvae and nymph) and highly mobile preys (e.g. Araneae and Formicidae), being the last one the most recorded (see Schoener 1971; Huey & Pianka 1981; Rocha *et al.* 2009). The high number of ants present in the stomach contents of the three species is partially explained

by the fact that this arthropod may constitute an important source of food in small islands, due to its limited available prey according to Pérez-Mellado & Corti (1993). Furthermore, in mainland the feeding on ants is not considered by several authors a so important item (Huey & Pianka 1977; Castanzo & Bauer 1998; Wymann & Whiting 2002), since the availability and variety of food is higher. It was expected more records of Isopoda preys, only one was recorded, since they were one of the most referred in a similar diet studies with *Trachylepis affinis*, *Trachylepis maculilabris* and *Lepidothyris fernandii* (Akini *et al.* 2002; Eniang *et al.* 2014); in addition, isopods were observed in high quantities in the field trip (pers. obs.). Chilopoda was another arthropod observed in large numbers in the islands studied (pers. obs.), but was recorded in low quantities in the stomach contents analyzed. Other diet studies in African Scincidae show the low consumption of Chilopoda (e.g. Akini *et al.* 2002; Wymann & Whiting 2002; Eniang *et al.* 2014), which could mean that these arthropods are not the preferred food of the skinks. Contrary to what was expected, due to field observation the Isoptera order (e.g. termites) were not recorder in any of the species studied. Although, termites seem to be an important source of food for numerous African lizard species (Bauer *et al.* 1989; Mouton *et al.* 2000), *Trachylepis* species included (e.g. *T. affinis*: Akani *et al.* 2002; *T. margaritifer*: Wymann & Whiting 2002; *T. atlantica*: Rocha *et al.* 2009), and with other genus (e.g. *Brasiliscincus agilis*: Vrcibradic & Rocha 1995, 1996; Vrcibradic 2001; *Mabuya striata wahlbergii*: Heideman & Bates 1999), where these termites were a frequently consumed prey.

The *Trachylepis a. adamastor* diet included 13 prey types and the most important items were Acari (145 items; present only in this species), Formicidae (15 items) and Araneae (10 items). The high number of Acari can be explained by the abundance of bird nests in the Tinhosa Grande islet, and possibly were consumed opportunistically, however, further studies are needed to confirm this. Ineich *et al.* (2009) observed the presence of Acari in stomach contents of *Trachylepis wrightii,* which inhabit in the Fregate Island, also with a very dense number of nesting birds. Despite this, the consumption of Acari is not very common in reptiles, which gives the idea that they are consumed mainly by opportunistic species (McAllister 1987; Ineich *et al.* 2009), which seems to be the case of *T. a. adamastor*.

The hand of one *T. a. adamastor* found in the stomach contents of one individual may have been due to an event of cannibalism. Although cannibalism is not common, it

was already recorded in other lizard species as *Trachylepis atlantica* (Silva *et al.* 2005) and *Hemidactylus mabouia* (Zamprogno & Teixeira 1998; Bonfiglio *et al.* 2006). Other items as lizard skin were recorded in some studies (Craig *et al.* 2007) but these could represent the ingestion of its own sloughed skin rather than predation. Given the relative scarcity of information on factors that can induce cannibalism, this should be a subject to study in the future.

Trachylepis a. principensis presented 11 prey types in their contents, and the most recorded prey items were Hymenoptera (9 items), Homoptera (9 items), Formicidae (9 items) and Araneae (6 items). The low quantity of prey items is partially explained by the low amount of individuals of *Trachylepis a. principensis* used in this study. The *Trachylepis thomensis* has the lowest number of prey types, compared with the other two *Trachylepis* species. The arthropod groups most recorded prey of *Trachylepis thomensis* were Diptera (15 items), Coleoptera (7 items and several undetermined items), Araneae (4 items and several undetermined items) and Formicidae (4 items). More data is however needed to better understand the diversity and patterns of preys consumed by these species. The gathering of this new data should be done on a series of specimens collected over an extensive time frame in order to test for differences of prey consumption between different times of the year (see Rocha *et al.* 2004; Craig *et al.* 2007; Rocha *et al.* 2009) or also collect fecal material (see Williams *et al.* 2015).

Despite the islets are integrated in the Biosphere Reserve of Príncipe and considered a RAMSAR site, the Tinhosa Grande is visited occasionally by local fishermen for fishery and use the land to dry the fish (Nuno Barros pers. comm.). According to present knowledge, fishermen do not present a direct threat to *T. a. adamastor* (Ceríaco 2015). Although there were no known natural predators of *T. a. adamastor* in the Tinhosa Grande islet, the introduction of cats (*Felis catus*), dogs (*Canis* spp.) and/or rats (*Rattus* spp.) by local fishermen intentionally or not, can lead to declining population (Ceríaco 2015). Silva *et al.* (2005) reported the predation by introduced species like rats and domestic cats of *T. atlantica*. The introduction of these predators has caused drastic declines and extinctions of reptilian species endemic to islands (Iverson 1978; Case & Bolger 1991; Daltry *et al.* 2001; Powell & Henderson 2005). Given the limited nature of the data presented here new studies are necessary to better understand the size of the population using more adequate methods and longer

prospects, as well as the collection of more specimens for the analysis of the diet.

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Appendix I - Number of arthropods found in the stomach contents of the specimens of *T. a. adamastor*, *T. a. principensis* and *T. thomensis* (N=26). Arthropods are ordered by the taxonomy, from left to right from highest to lowest rank.

Taxon	Specimen	Phylum: Arthropoda	Class: Chilopoda	Class: Symphyla	Subclass: Acari	Order: Araneae	Order: Coleoptera	Order: Coleoptera larvae	Order: Collembola larvae	Order: Dermaptera	Order: Diptera	Order: Diptera larvae	Order: Hymenoptera	Order: Isopoda	Order: Lepidoptera larvae	Order: Mantodea	Order: Thysanoptera	Order: Thysanura	Suborder: Caelifera	Suborder: Homoptera	Suborder: Homoptera nymph	Family: Curculionidae	Family: Formicidae	Family: Tettigoniidae
T. a. adamastor	MB03-001015				103												1							
	MB03-001016		1			1				3													1	
	MB03-001017																							
	MB03-001018	1			4	1																	2	
	MB03-001019			1	2																			
	MB03-001043			1	1																			
	MB03-001045		3		20	4	1	1	1												1		1	
	MB03-001046				2										1								2	
	MB03-001047				4																		8	
	MB03-001048				6	2												1						
	MB03-001049				1	2																		
	MB03-001050				2																		1	
T. a. principensis	MB03-001003					5	1				1		1						1				8	1
	MB03-001004						1				1												1	
	MB03-001005												9							9				
	MB03-001021		1			1																		
	IICT73- 1955					2																1		
T. thomensis	IICT3- 1954											15												
	IICT4- 1954					3																		
	IICT5- 1954					1																		
	IICT6-1954					*					1					1								
	IICT7-1954					3	1																2	
	IICT13- 1954					*																	1	
	IICT14- 1954																						1	
	IICT15- 1954					3							1							1				
	IICT16- 1954												1							1				

*- undetermined number

Conclusion

After the accomplishment of the field work and the analysis of the morphology, molecular and diet results it was possible to deepen the knowledge about the Adamastor skink and have a first understanding of its ecology and interspecific relationships with the surrounding populations. The molecular analysis with the two mitochondrial (16S and ND2) and one nuclear (RAG-1) genes, contrary to what was expected showed that the species *T. adamastor* and *T. principensis* are genetically conspecific. These results entail conservation issues due to the fragility of the Tinhosa Grande population and the morphological differences between the two populations. Given that and according to the Principle of Priority of the ICZN Code the name *T. adamastor* is therefore applicable for the two populations, since it was the first to be described, and the interpretation of each population as subspecies makes that the Tinhosa Grande population needs to be called *T. adamastor adamastor*, while the Príncipe population becomes *T. adamastor principensis.*

The current study is the first to provide important data regarding *T. a. adamastor* estimated population density as well its dietary composition and its interspecific relationships on the Tinhosa Grande islet. Due to the logistics implications, the obtained results are deficient to achieve a solid conclusion about the population density and ecology. Additional surveys, as well the collection of new and fresh data will be essential to have a substantial information about these issues.

This study also reinforces the importance of combination of morphological and molecular data for description of new species, in order to give certainty and eliminate doubts on relations between species and intra-population diversity.

Although the two populations are conspecific, its different morphology and the isolation and conditions of the Tinhosa Grande islet, it is crucial to consider *T. adamastor adamastor* a threatened population. These different populations should be treated differently, and it is fundamental that future studies of ecology, abundance and threats, especially of introduced species, be carried out in order to understand the status of threat so that adequate conservation plans can be drawn up. Considering this it is urgent that the national authorities take action for the conservation of this population, since it is vulnerable to external perturbations as introduction of predators, environmental issues as climate change or others problematic incidents.

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