The herpetofauna (Amphibia, Crocodylia, Squamata, Testudines) of the Isalo Massif, Southwest Madagascar: combining morphological, molecular and museum data

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Abstract. This study summarizes the data of multiple surveys carried out at the Isalo Massif (southwestern Madagascar), both within and outside the National Park limits between 2004 and 2014, providing an updated list of the amphibians and non-avian reptiles present within the area. We used an integrative taxonomic approach, combining morphological and molecular data sets to identify collected samples as either described species, or as undescribed candidate species. We here report on one new amphibian candidate species (Mantidactylus sp. aff. multiplicatus Ca65 “Isalo”), and two new amphibian distribution records (Mantella sp. aff. expectata “South”, Scaphiophryne sp. aff. calcanata Ca02) compared to the former inventories of the area. Additionally, we provide molecular reference sequences for 23 of the 24 amphibian taxa and for 40 of the 47 reptile taxa known to occur in the area, combining all available sources. With this study, we validate Isalo as an important centre of biodiversity and micro-endemism within Madagascar, with four described endemic amphibian (Gephyromantis azzurrae, Mantella expectata, Mantidactylus noralottae, and Scaphiophryne gottlebei) and one reptile species (Trachylepis nancycoutae). Of the taxa here listed for Isalo, seven amphibians and six reptiles are new candidate species, and among them at least one amphibian (Mantidactylus sp. aff. multiplicatus Ca65 “Isalo”) and three reptiles (Lygodactylus sp. aff. tuberosus Ca02 “Isalo”, Paroedura sp. aff. bastardii Ca02 “Isalo” and P. sp. aff. bastardii Ca03 “Ilakaka”) are currently known only from Isalo. The unique biodiversity of the Isalo Massif was likely favoured by the confluence of different biomes in the area and/or its role as a refugium during palaeoclimatic oscillations. Our study highlights the crucial importance of applying molecular taxonomic identification in updating the species lists even of previously well surveyed areas, and of maintaining and increasing museum voucher collections as a permanent and accessible testimony of the biodiversity inventory.

Key words. Dry forest, museum specimens, species survey, integrative taxonomy, candidate species, 16S rRNA, COI.

Introduction

Madagascar is one of the most important hotspots of biodiversity worldwide (Myers et al. 2000, GOODMAN & BENSTEAD 2005, KREMEM et al. 2008, VIETTES et al. 2009, Aymoz et al. 2013). The island is characterized by an incredibly high faunal endemism rate, ranging from 50% in birds to 100% in native amphibians (GLAW & VENCES 2007, PERL et al. 2014), and with about 400 non-marine squamate species only found in Madagascar (GLAW & VENCES 2007, JENKINS...
et al. 2014). Madagascar is characterized by a strong climatic heterogeneity and it is roughly divided from north to south into a humid east and an arid west (Vences et al. 2009, Brown et al. 2016). Five broad bioclimatic zones are currently recognized: the eastern humid rainforest belt, the sub-humid highlands, the western dry deciduous forests, the southwestern sub-arid spiny forests, and the montane grasslands and shrublands (Schatz 2000, Ganzhorn et al. 2001, Glaw & Vences 2007, Vences et al. 2009). The climate in the western part of the island is overall hot and dry, with a cooler-drier and a warmer-wetter season that each last 5–7 months (Goodman & Benstead 2003). This climate supports a diversity of sclerophyllous deciduous forests, open woodland and savannas that change in composition from northern subhumid areas to southern spiny bushland (Goodman & Benstead 2003). Diversity patterns of the herpetofauna largely reflect this subdivision, with bioclimatic boundaries coinciding with major areas of biotic turnover (Brown et al. 2014, 2016). Dry deciduous forest in Madagascar has been experiencing a dramatic decrease in the last decades and, since the 1970s, has been reduced by almost 40% of its original extent (Waeber et al. 2015). Knowledge is limited on the fauna and flora of this biome, and even less is known about their biology and ecological interactions.

While the richness of amphibians peaks in the eastern and northern rainforest areas (e.g., more than 100 amphibian species are known to occur in Ranomafana National Park, 80 in Betampona Strict Nature Reserve, and at least 70 in Marojejy National Park; Vieites et al. 2009, Rosa et al. 2012, Goodman & Wilmé 2003), the reptiles also exhibit high species richness in western and southwestern Madagascar (Raselimanana 2008, Brown et al. 2016). In contrast, the patterns of local endemism appear to be distinctly higher, for both amphibians and reptiles, in the north (D’Cruze et al. 2008, Brown et al. 2016).

An analysis of the herpetofaunal diversity of the dry forests of western Madagascar (Raselimanana 2008) has confirmed this bioregion as an important centre for species diversity and local endemism. At least 30% of the island’s named herpetofauna occurs in this region, with areas of higher habitat heterogeneity reaching distinctly higher species richness values (Raselimanana 2008, Bora et al. 2010, Gardner et al. 2015, Brown et al. 2016). For example, in the Tsingy de Bemaraha Massif, 19 species of amphibians and 60 of reptiles have been recorded, and of these, five amphibians and 18 reptile species are considered local endemics (Bora et al. 2010).

One other important area known to be highly heterogeneous in terms of available habitats and hosting many local endemic species is the Isalo Massif (Mercurio et al. 2008). Part of this area is included in the Parc National de l’Isalo, established in its current borders in 1962 (Goodman & Benstead 2003). In this study we update the previously published inventory of the amphibians of this area (Mercurio et al. 2008), and report on the known reptiles (Squamata, Testudines and Crocodylia) inhabiting this area, complementing the former, unpublished inventory on the herpetofauna of Isalo by Hawkins (1994). We used an integrative taxonomic approach, combining the external morphology of specimens, photographs in life, and DNA sequences to assign collected/observed amphibians and reptiles to either described species or undescribed candidate species. To allow these data to be as informative as possible for a better management of the area’s unique biodiversity, we also provide a list of the available voucher specimens hosted in the herpetological collection of the Museo Regionale di Scienze Naturali di Torino (Italy). The term ‘reptiles’ is used in this study as referring to non-avian reptiles only, i.e., in this case vertebrates in the orders Squamata, Testudines and Crocodylia.

Material and methods

Study site

The Isalo Massif is situated in the southwestern corner of the Province of Fianarantsoa (Fianarantsoa Faritany) and hosts the Parc National de l’Isalo, one of the largest protected areas of Madagascar (81 540 ha). Elevation ranges between 510 and 1268 m, with narrow canyons of up to 200 m deep, most of which occur in the eastern and southwestern sectors of the area. This area is characterized by the presence of sandstone outcrops and rocks with a mosaic of three main habitat types: savannas, open valleys, and narrow canyons (Mercurio & Andreone 2006). Savannas are expansive prairies that are repeatedly exposed to fire during the dry seasons, with isolated trees and forested spots. Humidity is low and temperature fluctuations between night and day are high. In this kind of habitat, rivers and pools are usually temporary, fed by seasonal rains, and dry for most of the year. The open valleys are usually vegetated by gallery forests that vary in dimension. Here the aquatic habitats consist of permanent or semi-permanent torrents with both cascades and pools being present. The montane, rocky part of the massif is intersected by canyons of different dimensions and depths in which water is present to a variable extent. In these humid canyons, vegetation is absent or limited to few isolated trees due to the scarcity of light. The climate is dry tropical, with 90% of precipitation occurring between November and March and with mean temperatures varying between 17°C in June and 25°C in February (Mercurio et al. 2008).

Survey methods

Multiple surveys were carried out in Isalo, both within and outside the National Park limits, between 2004 and 2014 (Fig. 1, Supplementary Table S1). Most of our sampling took place during the rainy season, when the activity of most species is at its highest. Sampling was conducted opportunistically during both day and night, using headlamps and hand-held torchlights, in a quest to explore all available habitats along transects or trails and by visiting seasonal streams and temporary ponds. Our search for amphibians
also included locating vocalising males. Locality data were recorded using a GPS receiver (Supplementary Table S1).

Most animals were photographed with analogical and/or digital cameras to document their coloration in life and subsequently released at the place of capture. For molecular analyses, toe clips of adult amphibians, fin tips of tadpoles, and tissue samples of reptiles were usually collected and stored in 99% ethanol. If individuals were suspected of possibly belonging to new and undescribed taxa, a limited number of specimens were collected as vouchers. They were anaesthetized by immersion in (amphibians), or injection with (reptiles) MS222, followed by euthanasia with an overdose of MS222. They were then fixed in 10% formalin or 90% ethanol and later transferred to 65–70% ethanol for long-term storage. The lists of tissue samples and voucher specimens analysed for this study are provided in Supplementary Tables S2–S5.

Species identification in the field was carried out mostly using the descriptions provided by Glaw & Vences (2007), and additional photographic records from scientific publications. The final inventory of amphibian and reptile species of the Isalo Massif is given as a compilation of the work carried out by our team and complemented by records retrieved from other publications for species not found by us (Glaw & Vences 1994, Hawkins 1994, Puente et al. 2005, Crottini et al. 2008, Mercurio et al. 2008).

Toponyms used in this study are those more consistently used in the literature (Supplementary Table S1). In most cases, anyhow, they correspond to informal local names.

Molecular species identification

Total genomic DNA was extracted from tissue samples using proteinase K digestion (10 mg/ml concentration) followed by a standard high-salt extraction method (Bruford et al. 1992). A fragment of ca. 550 bp of the 3' terminus of the mitochondrial 16S rRNA gene (hereafter referred to as 16S), proven to be suitable for amphibian identification (Vences et al. 2005a), was amplified for 159 samples, while a fragment of around 650 bp of the standard barcoding region of the cytochrome oxidase I gene (COI; Nagy et al. 2012) was amplified for 73 reptile samples. For three amphibian samples, it was not possible to amplify the 16S fragment, and in these instances other mitochondrial gene fragments were used, i.e., 16S 5' terminus, or cytochrome b (Cytb). The molecular taxonomic identification using the COI fragment was not possible for some reptile species due to missing reference sequences. In these instances, other mitochondrial gene fragments (16S, ND1, ND2, ND4, Cytb) were used for identification (see Supplementary Table S2 and S4 for details and GenBank accession...
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Vieites, Kumar 2007, specimens in Isalo have been deposited in the herpetological collection numbers of newly determined sequences [KX066545–KX066697, MH063278–MH063431], and Supplementary Table S6 for primers and cycling protocols). All fragments were sequenced using an ABI 3730XL automated sequencer at Macrogen Inc. Chromatograms were checked and sequences were manually edited where necessary, using the sequence alignment editor of BioEdit (V.7.2.0; Hall 1999).

To assess taxon identity, sequences of each morphologically identified taxon were compared among each other and each sequence was compared using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi, Altschul et al. 1997) with all homologous sequences available in GenBank http://www.ncbi.nlm.nih.gov/GenBank/, using the nucleotide blast (nblast) option with default parameters. Average genetic distances within and between species were calculated by uncorrected pairwise genetic distance estimations (uncorrected p-distance) using MEGA 7 (Kumar et al. 2016; Supplementary Table S7 and S8).

Some specimens could not be assigned to any described or identified candidate species (Vieites et al. 2009, Perl et al. 2014, Nagy et al. 2012). For these taxa, we applied the terms and abbreviations, confirmed candidate species (CCS), unconfirmed candidate species (UCS), or deep conspecific lineage (DCL) as defined by Vieites et al. (2009). Our denominations of already identified candidate species follow Perl et al. (2014) for amphibians and Nagy et al. (2012) for reptiles, although we decided to complement these working names with information on the morphologically closest described species by prefixing the species epithet with “sp. aff.” and a descriptor of the locality from where the taxon is known (this toponym was added in cases of species known only from Isalo) or of a characteristic trait of the candidate species.

Candidate species of amphibians were identified based on a threshold of 4% minimum divergence for the 16S gene fragment (see also Vences et al. 2005b, Fouquet et al. 2007, Vieites et al. 2009). Candidate species of reptiles were identified following the different thresholds proposed for the different groups by Nagy et al. (2012). The sequences of the other gene fragments were used to refine species identification within a known species group and there was no need to apply a minimum threshold.

In this study, we also provide updated distributional range information within the Isalo Massif for amphibians based on locality records from museum specimens, molecular data, GenBank, and literature (mostly Glaw & Vences 2007, Mercuroio et al. 2008; Supplementary Table S3). For reptiles, the distribution of each species includes locality records from museum specimens, molecular data, GenBank, and literature (mostly Glaw & Vences 2007, Hawkins 1994; Supplementary Table S5).

Museum specimens

Most of the specimens collected during our multiple surveys in Isalo have been deposited in the herpetological collection of the Museo Regionale di Scienze Naturali (Turin, Italy) and the Parc Botanique et Zoologique de Tsimbazaza (Antananarivo, Madagascar). We refer to these specimens using their collection numbers preceded by acronyms MRSN and PBZT, respectively. In addition to these, we provide taxonomic information on some additional specimens hosted in various collections. For these records, we use their catalogue numbers preceded by their institution acronyms (SMF, Senckenberg Museum Frankfurt, Germany; UADBA, Université d’Antananarivo, Mention Zoologie et Biodiversité Animale, Antananarivo, Madagascar; ZMA, Zoologisch Museum Amsterdam, The Netherlands (collections currently in the process of being integrated in the Naturalis Biodiversity Center of Leiden); UMMZ, Museum of Zoology at the University of Michigan; ZSM, Zoologische Staatssammlung München, Germany). Field number acronyms are FAZC (Franco Andreone Zoological Collection) and ACZCV (Angelica Crottini Zoological Collection Voucher). In instances where specimens have already been deposited in institutional collections, but are not yet catalogued, they are given as FAZC/MRSN (for specimens in Turin) and FAZC/PBZT (for specimens in Antananarivo), respectively.

Specimens hosted in the MRSN collection (Supplementary Table S3 and S5) were visually inspected and their taxonomic identification was reassessed based on the results obtained in this study. A careful morphological inspection and verification of diagnostic traits was performed when molecular data were insufficient to confirm species identity.

Results

In total, 24 amphibian and 47 reptile species were recorded in Isalo by combining all available data sources, and we here provide DNA sequences for almost all of these species. Like Mercuroio et al. (2008), we failed to detect in the field Scaphiophryne menabensis that, at the moment, remains known from the area by a single voucher specimen (UMMZ 227489, University of Michigan, Museum of Zoology: Vences et al. 2003, Glos et al. 2005).

Among the amphibians, 16 taxa (65%) belong to the family Mantellidae while six (26%) are Microhylidae. Hyperoliidae and Ptychadenidae are present with only one species each. We confirm the presence of 20 (out of the 21) amphibian species listed by Mercuroio et al. (2008) and provide evidence for the occurrence of one new candidate species (Mantidactylus sp. aff. multiplicatus Ca65 “Isalo”). We also report two new amphibian distribution records (Mantella sp. aff. expectata "South" and Scaphiophryne sp. aff. calcarata Ca02) compared to the previous inventories of the area. Four of the amphibian species and one candidate species seem to be Isalo endemics: Gephyromantis azzurrae, Mantella expectata, Scaphiophryne gottlebei, Mantidactylus noralottae and M. sp. aff. multiplicatus Ca65 “Isalo”. In this study, Gephyromantis corvus is tentatively not considered microendemic to Isalo, because there is a
record of this species from the Makay Massif (Rakotondramary & Goodman 2011), even though this record is in need of confirmation and might turn out to represent a different species of the subgenus Phylacomantis. Finally, we report new locality records for ten amphibian taxa within the Isalo Massif: Scaphiophryne sp. aff. calcarata Ca01, Dyscophus insularis, Boophis occidentalis, Laliostoma labrosum, Blommersia sp. aff. wittei Ca05 “Isalo”, Gephyromantis corvus, G. azzurrae, Mantidactylus sp. aff. ulceroxus Ca14 “Isalo”, M. noralottae, and M. sp. aff. cowani Ca48 “small” (see Supplementary Table S3).

Our molecular and morphological analyses of specimens MRSN A5313 (part of the type series of G. azzurrae Mercurio & Andreone 2007) suggest this specimen was wrongly assigned to G. azzurrae and we here propose to refer it to G. corvus (Fig. 4, Supplementary Table S2 and S3). This specimen was not analysed genetically by Mercurio & Andreone (2007) when describing G. azzurrae and was erroneously assigned to this species.

The reptile species of Isalo belong to 10 of the 15 families known from the island (Supplementary Table S4 and S5). Most abundant are Gekkonidae and Lamprophiidae, which are represented by 13 and 10 species, respectively (or 27 and 21% of the total). Isalo represents an expansion of the known range for six taxa (Crocodylus niloticus, Boophis occidentalis, Laliostoma labrosum, Blommersia sp. aff. wittei Ca05 “Isalo”, Gephyromantis corvus, G. azzurrae, Mantidactylus sp. aff. ulceroxus Ca14 “Isalo”, M. noralottae, and M. sp. aff. cowani Ca48 “small” (see Supplementary Table S3).

Species accounts

Amphibians

Mantidactylus (Brygoomantis) sp. aff. multiplicatus Ca65 “Isalo” (UCS) Fig. 2A

Locality records: Andohasahenina (MRSN A2964), Andriamane-ro (MRSN A6893, MRSN A6885, ACZCV 0281–0284), Namazaha Valley (also referred to as Namaza) (MRSN A2689, MRSN A2878, MRSN A6889, MRSN A6901), Piscine Naturelle.

This undescribed species represents a new record for Madagascar and could represent a new example of micro-endemism in Isalo. This lineage is phylogenetically close to M. multiplicatus, M. sp. aff. betsileanus Ca30 “Andohahela”, M. sp. aff. betsileanus Ca30 “Andohahela”, and the sympatric M. noralottae. Note that a formal resurrection of M. multiplicatus Boettger, 1913 is still needed, but this species name was already used by Poth et al. (2012) anticipating an upcoming taxonomic revision. The genetic distance between M. sp. aff. multiplicatus Ca65 “Isalo” and all the other species of the aforementioned group is ca. 4% (165). This UCS represents the third Brygoomantis from Isalo, with the other two being M. noralottae and M. sp.

...
aff. ulcersus Ca14 “Isalo”. New surveys followed up with molecular taxonomic identification of the samples will be needed to define its extent of occurrence in Isalo due to its superficial morphological similarity with M. noralottae.

Scaphiophryne sp. aff. calcarata Cao2 (CCS)  
Fig. 2B

Locality record: Ranohira (MRSN A6895).

The name S. calcarata (Mocquard, 1895) has not yet been unequivocally assigned to any population of Scaphiophryne, and three deeply divergent mitochondrial lineages exist in Madagascar: S. sp. aff. calcarata Cao1 (or S. sp. aff. calcarata B; known from Tolagnaro and Berenty), S. sp. aff. calcarata Cao2 (or S. sp. aff. calcarata A; known from Kirindy and Menabe) (terminology follows Vieites et al. [2009] and Perl et al. [2014], respectively), and S. sp. aff. calcarata Ankarafantsika (Perl et al. 2014). S. sp. aff. calcarata Cao1 was previously reported as S. calcarata by Mercuroio et al. (2008), and we here also report the occurrence of S. sp. aff. calcarata Cao2 (16S p-distance between populations from Kirindy and Isalo ca. 1%), revealing Isalo as a contact zone between these two taxa. The molecular divergence between S. sp. Cao1 and S. sp. Cao2 (ca. 4%; 16S) is supported by bioacoustic data (calls from Kirindy and Berenty are highly divergent, GLAW & VENCES [2007]), but no calls are known from Ankafantsika or Isalo. The population from Ankafantsika shows a 2% genetic dis-
This species is the most common freshwater turtle in Madagascar and widely distributed from the Sahamalaza Peninsula in the northwest (Penny et al. 2017) to all of the southwest. Pelomedusa subrufa is generally found in stagnant water bodies and temporary ponds. In Isalo, it was found in Ambatovaky and Zahavola in slow-flowing streams, and it was previously reported from here by Hawkins (1994).
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*Brookesia brygooi* belongs to the *Brookesia decaryi* group, which includes aridity-adapted species that live within the dry-deciduous forests on the western slope of the central high plateau of the island (Townsend et al. 2009). It inhabits the leaf litter stratum during the day and is found resting on low vegetation during the night. It can be quite difficult to observe in Isalo, although there is a record of one event in which several individuals were sampled in one single night (S. Ndriantsoa, pers. comm.) This population is genetically similar to the populations of *B. brygooi* at Kirindy with which it shares a distinctive lineage when compared to *B. brygooi* from Ankaraantsika (Crottini et al. 2012).

*Furcifer major* (Brygoo, 1971)
Fig. 6B

Locality records: Andranomena (MRSN R2891), Malaso, Ranohira (MRSN R1185), Zahavola (MRSN R2474–R2475), Isalo (no precise locality).

This taxon was formerly considered a large-sized subspecies of *Furcifer lateralis* (Brygoo 1971, Klaver & Böhme 1997) inhabiting the arid southwestern region of Madagascar. A recent taxonomic revision of the carpet chameleons elevated this taxon to species rank (Florio et al. 2012), which therefore also applies to specimens from Isalo. Hawkins (1994) reported this taxon as *F. lateralis*. The samples analysed in this study are genetically similar to each other (Supplementary Table S8) whereas they have a genetic distance at the COI fragment of ca. 2–3% to *F. viridis*, 3–4% to *F. lateralis*, and ca. 9–10% to sympatric *F. oustaleti* and *F. verrucosus*.

*Furcifer oustaleti* (Mocquard, 1894)
Fig. 6C

Locality records: Analalava Forest, Bereketa (MRSN R3161), Namazaha Valley.

A recent phylogeographic analysis revealed the presence of two distinctive lineages within this species, but refrained from formally describing a new species based on the still-limited evidence available (Florio & Raxworthy 2016). The populations from Isalo belong to *F. oustaleti* Clade D sensu Florio & Raxworthy (2016) and are genetically uniform for the marker analysed (Supplementary Table S8).

*Furcifer verrucosus* (Cuvier, 1829)
Fig. 6D

Locality records: Piscine Naturelle, Sakavato (MRSN R3165, MRSN R3169).

Slightly smaller, *F. verrucosus* can be easily mistaken for *F. oustaleti* that also widely overlaps its distribution range. *Furcifer verrucosus* is quite common in disturbed areas although it can be found in a wide array of habitats at sites below 120 m a.s.l. (Raselimanana & Rakotomalala 2003). A recent phylogeographic analysis revealed the presence of a western and an eastern lineage (Florio & Raxworthy 2016). The Isalo population belongs to *F. verrucosus* Clade B sensu Florio & Raxworthy (2016), and the analysed samples from Isalo exhibit a genetic variability of 0.7% (COI; Supplementary Table S8) between each other.

GEKKONIDAE

*Blaesodactylus sakalava* (Grandidier, 1867)

Locality record: Isalo (no precise locality).

In Isalo, this species is currently known from a record made by Hawkins (1994) and a photographic record available online (flickriver, Pete Read: http://www.flickriver.com/photos/128237756@No6/1983428834/). No molecular data from Isalo are available for this species.

Figure 5. Chelonian and a crocodilian species found in the Isalo Massif: A) *Pelomedusa subrufa* (MRSN R3737) from Zahavola in life; B) *Crocodylus niloticus* preserved specimen (FAZC 14784, PBZT-uncatalogued) from Hazofotsy River (Ranohira).
Figure 6. Photos in life of lizard species recorded in the Isalo Massif: A) Brookesia brygooi (ACZC 2569) from the Namazaha Valley; B) Furcifer major (FAZC 14640) from Zahavola; C) Furcifer oustaleti from the Analalava Forest (photo not reliably referable to a certain sample or voucher); D) F. verrucosus from circuit Piscine Naturelle (photo not reliably referable to a certain sample or voucher); E) Hemidactylus mercatorius (ACZC 1945) from the Namazaha Valley; F) Lygodactylus sp. aff. tuberosus Ca02 (MRSN R3746) from Malaso; G) Paroedura picta from Zahavola (photo not reliably referable to a certain sample or voucher); H) P. sp. aff. bastardi Ca01 (FAZC 14631) from Zahavola; I) P. sp. aff. bastardi Ca03 from Ilakaka (photo not reliably referable to a certain sample or voucher); J) P. vahiny (MRSN R3748) from Malaso; K) Phelsuma hielscheri from Piscine Naturelle (tissue sample not collected); L) P. mutabilis from the Namazaha Valley (tissue sample not collected); M) P. standingi from Isalo (no precise locality, tissue sample not collected); N) Zonosaurus laticaudatus (MRSN R3213) from the Canyon des Makis; O) Z. madagascariensis from Andranonombilahy (tissue sample not collected); P) Z. karsteni from Analamangabe (tissue sample not collected); Q) Chalarodon madagascariensis from Ilakaka (tissue sample not collected); R) Oplurus cyclurus from Malaso (photo not reliably referable to a certain sample or voucher); S) O. quadrivinculatus from Zahavola (tissue sample not collected); T) O. saxicola (photo not reliably referable to a certain sample or voucher) from Sakavato; U) Trachylepis elegans from the Namazaha Valley (tissue sample not collected); V) T. gravenhorstii from the Namazaha Valley (tissue sample not collected); W) T. nancycoutue from the Namazaha Valley (tissue sample not collected).
Geckolis typica Grandidier, 1867

Locality records: Analalava Forest (UMMZ 215902–215903), Beraketa (UMMZ 196575–196576), Sahana (UMMZ 215904), Isalo (no precise locality).

The only specimens of this taxon from Isalo are those in the collection of the Museum of Zoology at the University of Michigan, but another record stems from Hawkins (1994). As for Isalo, neither molecular data nor information on its habitat are available.

Hemidactylus mercatorius Gray, 1842

Fig. 6E

Locality records: Analalava Forest (MRSN R2406–R2407), Malaso (FAZC 14650/PBZT-uncatalogued), Namazaha Valley, Ranohira – Motel d’Isalo, Zahavola.

Hemidactylus mercatorius is one of the few non-endemic squamate species of Madagascar, being present and widely distributed in eastern Africa and on many islands of the Indian Ocean. Samples from Isalo belong to the most common haplotype, occurring both in the Sambirano and northeast, and in the southern central east, southeast, and south (Vences et al. 2004). The analysed populations exhibit an average genetic variation of 2.6% (COI; Supplementary Table S8) between each other.

Lygodactylus sp. aff. pictus Ca01 “Isalo” (UCS)

Locality record: Analalava Forest (ZMA 19595).

This genus still contains several undescribed species (Puente et al. 2005, 2009, Mezzalama et al. 2017). Isalo lineage was previously reported by Puente et al. (2005) and refers to a specimen deposited in the collection of the Naturalis Biodiversity Center, Leiden (The Netherlands), collected in the Analalava Forest. It is morphologically similar to L. pictus although genetically it has at least 11% genetic distance to all other species of Lygodactylus for which 16S sequences are currently available. For comparison, we sequenced the 16S and COI fragment (16S: MH063336–MH063337, COI: MH063413–MH063414) of a recently collected specimen of Lygodactylus sp. aff. pictus Ca01 “Isalo” from the Forêt de Vohibasia (Zombitse-Vohibasia National Park) and the 16S fragment proved to be 99% identical to the 16S sequence of this taxon collected in the Analalava Forest (Puente et al. 2005; AY653238). This lineage is therefore currently known at least from Isalo and Zombitse-Vohibasia.

Lygodactylus sp. aff. tuberosus Ca02 “Isalo” (UCS)

Fig. 6F

Locality records: Malaso (MRSN R3746, MRSN R3749).

This candidate species is identified here for the first time. The analysed specimens were found in open grassland near canyon entrances and rocky microhabitat in Malaso. Morphologically, this taxon is quite similar to L. tuberosus. The genetic distance between L. tuberosus from Ifaty (MH063412) and this UCS from Isalo is ca. 17% at the analysed COI fragment. The genetic distance in 16S between this UCS and the syntopic Lygodactylus sp. aff. pictus Ca01 “Isalo” is ca. 10%, and ca. 12% to L. tuberosus from Toliara and Belalanda (e.g., AY653283, LN998658).

Paroedura picta (Peters, 1854)

Fig. 6G

Locality records: Andriamanerana, Zahavola.

This ground-dwelling species is typically found in the dry forests or on sandy substrate in spiny scrub vegetation of southern Madagascar (Glaw & Vences 2007). Within Isalo, it is commonly encountered in open savannah areas. The samples here analysed have a genetic distance of 6% (16S) to P. picta from Kirindy (AF215242) and only 1% to P. picta from the Toliara region (GU128988). In the COI fragment analysed, the samples of P. picta from Isalo have ca. 2–3% genetic distance to other P. picta from the pet trade or from Isalo (KR149293 and JQ909507, respectively), and ca. 13% distance to another P. picta from the pet trade (KMO47640), therefore suggesting the existence of a complex of cryptic species.

Paroedura sp. aff. bastardi Ca01 “Marofandilia/Miandrivazo” (UCS)

Fig. 6H; Fig. 8A

Locality records: Zahavola (MRSN R3736, MRSN R3745), Namazaha Valley (MRSN R2448), Isalo (no precise locality, MRSN R2553).

This UCS belongs to the P. bastardi species complex, which contains several undescribed species (Jackman et al. 2008, Aprea et al. 2013, GLaw et al. 2014). It was found within the canyon in a shaded area close to the stream where humidity was high due to the presence of a small cave and a waterfall. It was already known from Marofandilia and Miandrivazo, and the 16S genetic distance of the Isalo population to these other populations is 3–4% (GU129005, Marofandilia and GU128989, Miandrivazo; Aprea et al. 2013).

Paroedura sp. aff. bastardi Ca02 “Isalo” (UCS)

Locality records: Malaso, Zahavola (FAZC 14657/PBZT-uncatalogued).

This UCS also belongs to the P. bastardi species complex. In 2009, individuals belonging to this taxon were found on 187
The poorly known species is characterized by having an overall smooth appearance, determined by the presence of small and scattered tubercles and numerous small scales on both body and head. In his report, HAWKINS (1994) listed *P. androyensis* for Isalo National Park, but this record almost certainly refers to *P. vahiny*, described few years after. In contrast to the holotype of *P. vahiny* that was found on a plant stem in the forest, this taxon was found on the ground in Isalo. It exhibits a genetic distance, at the COI fragment, of ca. 20–24% to all the other syntopic species of *Paroedura* (Supplementary Table S8), but the lack of a COI reference for *P. vahiny* from the type locality prevents us from assigning these samples on a molecular basis. Similarly, for the 16S gene fragment, samples of this taxon are 81–82% similar to almost all other *Paroedura* species. We here refer to this taxon as *P. vahiny* pending confirmation by means of a molecular comparison with samples from the type locality.

**Paroedura vahiny**

*Phelsuma hielscheri* RÖSLER, OBST & SEIPP, 2001

![Fig. 6K](image)


*Phelsuma hielscheri* is a regionally endemic species of southwestern Madagascar that belongs to the *P. dubia* group (ROCHA et al. 2010). This species is so far known only from a few localities such as Kirindy, Morondava and Isalo. *Phelsuma hielscheri* is quite difficult to observe in Isalo. Individuals were spotted during the daytime, active on *Pan danus* sp. leaves. Only one specimen was collected in Zahavola from which no DNA sequences could be obtained.

**Phelsuma mutabilis** (GRANDIDIER, 1869)

![Fig. 6L](image)

Locality record: Namazaha Valley.

*Phelsuma mutabilis* is distributed along the southern and western Madagascar coasts up to Ankaranafantsika in the northwest (CROTTINI et al. 2011). This species is known to inhabit both natural and anthropogenic areas and is adapted to live in different environments such as trees and bushes, but also on walls of buildings or fences. In Isalo, a representative of this species was observed in the Namazaha Valley where it was basking on a tree trunk (A. CROTTINI, pers. obs.). *P. mutabilis* exhibits an intraspecific genetic distance of 4.7% across Madagascar (16S, CROTTINI et al. 2011). The analysed sample from Isalo confirms this intraspecific variability by showing a genetic distance ranging from 4 to 6% (16S) to other populations (Toliara, Ejeda, Antsalova: ROCHA et al. 2009; Beroroha, Makay: SOUND et al. 2006).

**Phelsuma standingi** METHUEN & HEWITT, 1913

![Fig. 6M](image)

Locality records: Ranohira, Isalo (no precise locality).

*Phelsuma standingi* is a large species of day gecko with unresolved phylogenetic relationships (ROCHA et al. 2009, 2010), occurring only in the arid spiny forest of southwestern Madagascar. Like *P. hielscheri*, *P. standingi* is quite rarely seen in Isalo and our surveys did not yield any new observations. The only DNA sequence available for this species from Isalo refers to a published record (16S, DQ270564), of which the locality is given as “South of Isalo” (SOUND et al. 2006). Therefore, it might actually refer to the Zombitse-Vohibasia National Park, where we have observed the species as well, rather than to the Isalo Massif itself. On the other hand, BERGHOFF & KRAUSE (1999) reported on a visual record of this species in Ranohira.
GERRHOSAURIDAE

Tracheloptychus madagascariensis Peters, 1854
Locality record: Isalo (no precise locality).

This taxon was reported from Isalo by Brygoo (1985). No molecular data from Isalo are available for this species.

Zonosaurus laticaudatus (Grandidier, 1869)
Fig. 6N
Locality records: Canyon des Makis (MRSN R3213), Isalo (no precise locality, FAZC 12767/MRSN R-uncatalogued).

In Isalo, this species was observed in forested habitat inside large canyons. The analysed samples suggest a close relationship to Z. laticaudatus from Tsingy de Bemaraha (6% for COI, JQ909628; 2% for the 16S fragment, KC515139; Nagy et al. 2012, Recknagel et al. 2013).

Zonosaurus madagascariensis (Gray, 1831)
Fig. 6O
Locality records: Andranonombilahy, Canyon des Makis (MRSN R3232).

In contrast to Z. laticaudatus, this species is mostly distributed in eastern Madagascar, although there are areas in the northwest where these two species co-occur. In Isalo, the observed individuals were found in forested habitat inside or at the entrance of large canyons. The samples analysed show a genetic distance of ca. 1–2% to Z. madagascariensis from Tsingy de Bemaraha (JQ909629 and Mahasoa (JQ909630), and 7% to specimens from Marojejy (JQ909631) (COI). At the 16S fragment, Z. madagascariensis from Isalo is only 1% divergent from the closest sister species, Z. haraldmieriei from the Forêt d’Ambre (KC515136). More in-depth analyses will be needed to assess whether Z. haraldmieriei is a chromatic variant of Z. madagascariensis or represents a true sister species of recent divergence (Raselimanana et al. 2009, Recknagel et al. 2013, Blair et al. 2015).

Zonosaurus ornatus (Gray, 1831)
Locality record: Isalo (no precise locality).

Not encountered during our surveys, but reported from Isalo by Glaw & Vences (1994), where it was observed in open grasslands. This visual record is in need of confirmation, as no photographic or physical voucher is available, and we cannot exclude this record was mistaken, e.g., by confusion with an unusually colored Z. karsteni. No molecular data from Isalo specimens are available.

Zonosaurus karsteni (Grandidier, 1869)
Fig. 6P
Locality records: Analamangabe, Ranohira Bas (FAZC 14698/PBZT-uncatalogued), Isalo (no precise locality).

This species is currently known from fragmented and geographically distant populations in the dry forests of western Madagascar (Glaw & Vences 2007). It has been reported before in Isalo by Hawkins (1994). The populations of this species analysed so far (Andranomanintsy, Bemaraha, Kirindy, Lambokely, Mikea, and Petriky) show a low phylogeographic substructure (Raselimanana et al. 2009). Zonosaurus karsteni from Isalo confirms this pattern in that the samples had a genetic distance at both the 16S and ND1 fragment of only 1% to Z. karsteni from Toliara and Mikea-Ankindranoka.

OPLURIDAE

Chalarodon madagascariensis Peters, 1854
Fig. 6Q
Locality records: Analalava Forest (UADBA 21059), Antambonoa (MRSN R3127–R3128), Canyon des Makis (MRSN R3219), Ilakaka, Vohimaro (FAZC 12792/PBZT-uncatalogued), Isalo (no precise locality, MRSN R2446–R2447).

This species was encountered in Vohimaro after it was already reported from this area (Hawkins 1994) and Analalava (Münchenberg et al. 2008). Samples from Isalo are genetically more similar to the population of C. madagascariensis at Tsingy de Bemaraha (in the central west) than to the geographically closer populations at Toliara (Münchenberg et al. 2008).

Oplurus cyclurus (Merrem, 1820)
Fig. 6R

Oplurus cyclurus is a largely arboreal and rupicolous iguana that can be easily identified by its tail bearing enlarged spines (Glaw & Vences 2007). In Isalo, specifically in the Analalava Forest, two mitochondrial lineages with a genetic distance of up to 2% at the 16S occur (Münchenberg et al. 2008). Our samples of O. cyclurus from Malaso are genetically uniform for both 16S and COI (Supplementary Table S8) and equally divergent (1%; 16S) from the samples of both clades present in the Analalava forest.

Oplurus grandidieri Morquard, 1900
Locality record: Antoha (MRSN R3235).
Only one specimen of *Oplurus grandidieri* was collected in Isalo. It exhibits a genetic distance of 2% to a sample of *O. grandidieri* from Zazafotsy (16S; EU997755; Münchenberg et al. 2008) and of 3% from two samples from the pet trade (COI, KF882905 – Altmanova et al. 2015; AB218720, Okaïma & Kumazawa 2009). *Oplurus grandidieri* has a low genetic divergence compared to *O. fierinensis* (16S, c-mos uncorrected p-distances = 2.0 and 0%, respectively; Münchenberg et al. 2008), and according to our data, the COI distance is 6% compared to a specimen of *O. fierinensis* from Toliara; JQ909485, Nagy et al. 2012.

### Oplurus quadrimaculatus Duméril, 1851

**Fig. 6S**

Locality records: Andohasahenina (MRSN R2380), Andranomena (MRSN R2378–R2379, MRSN R2381), Malaso (FAZC 14687/MRSN R-uncatalogued), Zahavola, Isalo (no precise locality, MRSN R2365–R2370).

This species lives throughout the arid areas of southwestern Madagascar, but can also be found in the drier parts of the central highlands up to 2050 m a.s.l. (Ibity Massif) and near the rainforest in the Tolagnaro area (Glaw & Vences 2007). In Isalo, it was collected in Malaso, but, in contrast to *O. cyclurus*, which is more arboreal, this relatively common species was found on large rocks and boulders. This taxon was previously recorded by Hawkins (1994). *Oplurus quadrimaculatus* is known to comprise two well-supported clades, one representing the highland populations (Antoetra, Ambositra, and Andringitra) and the other one the southern populations (Toliara, Tolagnaro, Andohanahela, and Tranoroa) (Münchenberg et al. 2008). The population at Isalo belongs to the southern lineage, and one sample exhibits a 1% distance (16S) to this southern lineage. At the COI fragment, *O. quadrimaculatus* from Isalo shows a genetic distance of 3% to samples from Andohanahela (JQ909486).

### Oplurus saxicola Grandidier, 1869

**Fig. 6T**

Locality record: Sakavato (MRSN R3243–R3246).

This rock-dwelling species inhabits the south of Madagascar (Glaw & Vences 2007). In Isalo, it has been found only in Sakavato, and this represents the first observation in this area. Like *O. quadrimaculatus, O. saxicola* appears to comprise two divergent lineages: one in the southeast (Esomony from where a new species of *Chalarodon* has recently been described; Miralles et al. 2015), and one in the southwest (Münchenberg et al. 2008). The sample from Isalo belongs to this last clade from the southwest, from which it differs by 2% (16S), while it exhibits 3% (16S) divergence from the sample from Esomony.

### SCINCIDAE

### Grandidierina rubrocaudata (Grandidier, 1869)

**Fig. 6U**

Locality record: Isalo (no precise locality).

This taxon was reported for Isalo by Brygoo (1981). No molecular data are available for this species from Isalo.

### Trachylepis elegans (Peters, 1854)

**Fig. 6V**

Locality records: Andohasosy (MRSN R3224), Andranombilahy (MRSN R3239), Andranomena (MRSN R2437), Malaso, Namazaha Valley, Ranohira – Motel d’Isalo, Vallée du Petit Nazareth (MRSN R2455), Zahavola (MRSN R3123), Isalo (no precise locality, MRSN R1173).

In Isalo, individuals were found in open grasslands and outside canyons, where they are often seen basking on rocky surfaces. A recent preliminary analysis of the genetic variability of this species across its distribution identified four genetic lineages (Vences et al. 2014). The analysed samples from Isalo are genetically homogeneous (0.3%, Supplementary Table S8) and belong to the most widespread lineage of *T. elegans*, the lineage A sensu Vences et al. (2014).

### Trachylepis gravenhorstii (Duméril & Bibron, 1839)

**Fig. 6W**

Locality records: Sambalalhy (Andriamanerana), Malaso (FAZC 1465/MRSN R-uncatalogued), Namazaha Valley (MRSN R2449), Ranohira, Zahavola (MRSN R3221).

*Trachylepis gravenhorstii* can be found both in the ground and on tree trunks (Glaw & Vences 2007), but not inside canyons. In Isalo, we identified two different lineages, 4a and 4b (sensu Vences et al. 2014), with a genetic distance at the analysed COI fragment of 7% between each other. As far as we know, lineage 4a was found only in the village of Ranohira, whereas lineage 4b occurred in at least two localities within the Massif (Malaso and Zahavola). Molecular taxonomic identification of the individuals collected in the Namazaha Valley was not possible. The presence of *T. gravenhorstii* lineage 4b in Isalo represents a new distributional record for this lineage.

### Trachylepis nancycoutuae (Nussbaum & Raxworthy, 1998)

**Fig. 6W**

Locality records: Andranomena (MRSN R2401), Bemenara, Malaso (FAZC 14632/PBZT-uncatalogued), Namazaha Valley, Zahavola (MRSN R3221).

This skink species is endemic to Isalo, inhabiting rocky substrates including large boulders and cliff faces. *Trachy-
lepis nancycoutuae was found in the southeastern sections of the Isalo NP and in the centre of the massif. This is a very secretive species, which seems to be quite abundant in this region, although it is difficult to collect. A phylogenetic analysis of the genus Trachylepis has recently been published and revealed that T. nancycoutuae probably is the sister species of an undescribed species inhabiting the area of Ambalavao and Andringitra (T. sp. aff. vato; Lima et al. 2013).

LAMPROPHIIDAE

Dromicodryas bernieri (Duméry, Bibron & Duméry, 1854) Fig. 7A

Locality records: Andranomangara, Andranomena (MRSN R2367), Lola (MRSN R3227), Malaso, Ranohira (MRSN R2366), Zahavola (MRSN R3226).

In Malaso, this snake species was encountered during the day on rocks close to a shallow canyon, while in Ranohira, it was found along a secondary road. The analysed samples from Isalo are genetically similar to each other (0.5% Supplementary Table S8) and to the individuals from Ifaty (1%, JQ909361, Nagy et al. 2012).

Ithycyphus ours Domergue, 1986 Fig. 7B

Locality records: Canyon des Makis (MRSN R3120), Anjofo, Namazaha Valley.

In Isalo, this species has been spotted in the Canyon des Makis, Anjofo, and in the Namazaha Valley where one adult individual was observed predating on an adult Furcifer oustaleti (Crottini et al. 2010). Irrespective of their morphological differences, I. oursi and I. miniatus are genetically poorly differentiated (ca. 3%, COI). The two analysed samples from Isalo are identical to each other in COI, but have a ca. 5% genetic divergence from a sample from Ranobe (JQ909394; Nagy et al. 2012).

Madagascarophis meridionalis (Domergue, 1987) Fig. 7C

Locality records: Andranomena (MRSN R2336), Canyon des Rats (MRSN R3741), Malaso (FAZC 14658/PBZT-uncatalogued, FAZC 14788/PBZT-uncatalogued), Namazaha Valley (MRSN R2334, MRSN R2339), Ranohira: Motel d’Isalo, Reine de l’Isalo (MRSN R3183), Tsitorina (FAZC 12807/MRSN R-uncatalogued), Vallée du Petit Nazareth (MRSN R2335), Zahavola (MRSN R3182), Isalo (no precise locality).

In Isalo, we found two distinct genetic lineages of this species with ca. 7% of genetic divergence at the analysed COI gene fragment. The sample from the Canyon des Rats belongs to a lineage also present in Ifaty, Ihosy and Ranomafana (Nagy et al. 2012, Ruane et al. 2016), whereas samples from Malaso, Ranohira and Zahavola belong to a lineage found also in Tsimanampetsotsa (Nagy et al. 2012). Both lineages are present in the Namazaha Valley. Hawkins (1994) recorded this taxon as M. colubrinus.

Mimophis mahfalensis (Grandidier, 1867) Fig. 7D

Locality records: Andranomena (MRSN R2363), Bemenara (MRSN R2351, MRSN R2352), Bereketa (MRSN R3176), Malaso, Ranohira, Zahavola.

Mimophis mahfalensis and the recently described M. occultus Ruane, Myers, Lo, Yuen, Welt, Juman, Futterman, Nussbaum, Schneider, Burbrink & Raxworthy, 2017 are the only Malagasy snakes that belong to the mostly African subfamily Psammophiinae (Ruane et al. 2017). In Isalo, M. mahfalensis can be found in dry forest, thornbush savannah, and anthropogenic habitats. The analysed samples from Isalo are identical to each other in the markers studied (Supplementary Table S8) and more similar to the individuals from Ifaty (1%, COI) than to those from Toilari (5%, COI). Using specimens from Isalo, Rosa et al. (2014) provided data on the internal anatomy of M. mahfalensis to demonstrate the presence of toxin-secreting glands, supporting the notion to classify the Psammophiinae as a subfamily of the Lamprophiidae (Pyron et al. 2013).

Leioheterodon madagascariensis Duméry & Bibron, 1854

Locality record: Bereketa, Isalo (no precise locality).

In Isalo, this species is currently known only from a report by Hawkins (1994) and from Bereketa (Glaw & Vences 2007), but no molecular data are available.

Leioheterodon modestus ( Günther, 1863) Fig. 7E

Locality records: Analalava Forest (FAZC 14695/MRSN R-uncatalogued), Andozoky (MRSN R3236), Canyon des rats (MRSN R3234), Lola (MRSN R3233), Namazaha Valley, Ranohira (FAZC 14704/PBZT-uncatalogued), Ranohira – Motel d’Isalo, Isalo (no precise locality).

This is a common species in Isalo and individuals were seen in open savannas, gallery forest, and in canyons. In Isalo, individuals of L. modestus have been recorded to predate upon the microendemic frog Scaphiophryne gottlebei (Rosa et al. 2010). Specimen FAZC 14695, collected close to the Analalava Forest, was identified by molecular means (COI) as Leioheterodon gayi Mocquard, 1905. We compared a COI sequence of this specimen with sympatric and allopatric pop-
ulations of *L. modestus* (ACZC 1965, FAZC 14668, FAZC 14704 from Isalo; FAZC 14701 from Zazafotsy, MHO63415; FAZC 14769 from Pont Bevilany, MHO63416) and found that the COI sequence of specimen FAZC 14695 had an average genetic distance of 15% to the otherwise similar COI sequences of the other 5 analysed samples of *L. modestus*. We therefore amplified the 16S fragment of this specimen and of FAZC 14704 from Isalo and those sequences were identical (99% genetic distance), suggesting a possible amplification of a nuclear COI pseudogene in the case of specimen FAZC 14695. However, these 16S sequences had a 97–98% genetic similarity to *Leioheterodon madagascariensis* (AY188061 from Nagy et al. 2003). Morphologically, these six specimens (four from Isalo and two from elsewhere, details provided above) are identical to each other and they all correspond phenotypically to *L. modestus*. We therefore think that there might be a case of mislabelling the 16S sequence AY188061 (Nagy et al. 2003) and suggest that this sequence actually refers to *L. modestus*.

**Liophidium vaillanti** (Mocquard, 1901)  
Fig. 7F  
Locality record: Zahavola (MRSN R3210).

This species was rarely observed in Isalo. The known individual from Zahavola has a coloration that is quite different from those from other localities (compare pictures of *L. sp. aff. vaillanti* "South" – which corresponds to this record – on page 435 and of *L. vaillanti* on page 433 in Glaw & Vences 2007). However, analysed samples of this species exhibit limited genetic differentiation [1–2% to *L. vaillanti* from Kirindy (DQ979995, EU394720; Cytb) and Tsimanampetsotsa (GQ913677; Cytb); 1% to *L. vaillanti* from Tsimanampetsotsa (GQ913674; 16S)]. Mercurio et al. (2006) observed that this species is at least partly ophiophagous.

**Lycodyras guentheri** (Boulenger, 1896)  
Fig. 7G  
Locality records: Zahavola (MRSN R3739), Isalo (no precise locality).

We have only one record of this species from Zahavola, where the individual was found in a shrub outside the canyon. As for *L. carleti*, no sequences were previously available, but with 212 ventral scales (all other *Lycodyras* species have more), 17 scales at midbody, 78 subcaudals and 8 supralabial scales, our inspection of specimen MRSN R3739 suggests this record being referable to this taxon (Vences et al. 2004, Nagy et al 2010). A record of *L. gaimardi* exists from the Isalo area (Hawkins 1994), but neither morphological nor precise locality information are available, so it could represent another record for *L. guentheri*. Geneti-

![Figure 7. Photos of snake species in life recorded in the Isalo Massif: A) Dromicodryas bernieri from Malaso (tissue sample not collected); B) Ithycyphus oursi (ACZC 1932) from Namazaha Valley; C) Madagascarophis meridionalis (FAZC 14638) from Malaso (sample not sequenced); D) Mimophis mahfalensis (FAZC 14677) from Malaso; E) Leioheterodon modestus from the Namazaha Valley (tissue sample not collected); F) Liophidium vaillanti (MRSN R3210) from Zahavola; G) Lycodyras guentheri (MRSN R3739) from Zahavola; H) Lycodyras sp. aff. pseudogranuliceps Ca01 "Andohahela" (MRSN R3216) from Antoha; I) Thamnosophis lateralis (ACZC 1928) from the Namazaha Valley; J) Acrantophis dumerili from Ilakaka (tissue sample not collected); K) Sanzinia volontany from the Namazaha Valley (tissue sample not collected); L) Madatyphlops arenarius (FAZC 14696) from near Ilakaka (sample not sequenced).
cally, this taxon seems to be closely related to *L. maculatus* from the Comoros (ZSM 38/2010) and Mayotte (ZSM 42/2010 and ZSM 665/2000) from which it has between 3 and 4% genetic distance at the analysed 16S gene, respectively, and 13% at the COI fragment.

*Lycodryas* sp. aff. *pseudogranuliceps* Ca01 "Andohahela" (CCS)

Fig. 7H

Locality record: Antoha (MRSN R3216).

This undescribed species of *Lycodryas* is already known from Andohahela in southeastern Madagascar (NAGY et al. 2010). Considering similar geographical patterns in *Lio­phidium vaillanti* and *Mantella* sp. aff. *expectata* "South", this affinity of Isalo to Andohahela in southeastern Madagascar is interesting to note. In Isalo, this species was found in a small shrub at night. It seems to be closely related to *L. granuliceps* and *L. pseudogranuliceps*. The genetic distance between the specimen from Isalo and Andohahela is 1 and 5% at the analysed 16S and COI gene fragments, respectively. Our sample has 2% (16S) and 9% (COI) divergences from *L. pseudogranuliceps* from Befandriana-Avaratra in northern Madagascar (ZSM 193/2009, NAGY et al. 2010, 2012).

*Pseudoxyrhophus quinquelineatus* (GÜNTHER, 1881)

Locality record: Isalo (no precise locality).

This species is currently known from Isalo only by the record published by HAWKINS (1994). No molecular data from Isalo are available.

*Thamnosophis lateralis* (DUMÉRIL, BIBRON & DUMÉRIL, 1854)

Fig. 7I

Locality records: Andriamanerana (FAZC 12632/MRSN R-uncatalogued), Namazaha Valley.

The individuals found in the Namazaha Valley and Andriamanerana were active on trails during the day. The analysed samples from Isalo are quite similar to each other (0.8%, Supplementary Table S8) and genetically similar (3%; COI) to the individuals from Ranomafana as published by NAGY et al. (2012).

**SANZINIIDAE**

*Acrantophis dumerili* JAN, 1860

Fig. 7J

Locality records: Andohasahenina, Ilakaka, Namazaha Valley, Isalo (no precise locality).

This species was reported from Isalo by HAWKINS (1994). We observed it in Andohasahenina and Ilakaka, but no samples were collected. This species was recently (October 2017) found in Namazaha Valley (*L. Buffa*, pers. comm.), inactive on the ground during the day. Two species are currently known in this genus, but molecular analyses revealed a third lineage in the south and southeast of Madagascar (*OROZCO-TER WENGEL* et al. 2008). The only data for this species from Isalo available to us are those of a published record (16S, EU419793; OROZCO-TER WENGEL et al. 2008).

*Sanzinia vontanany* VENCES & GLAW, 2004

Fig. 7K

Locality records: Canyon de Zahavola, Namazaha Valley, Isalo (no precise locality).

This taxon was formerly considered a subspecies of *S. madagascariensis* (OROZCO-TER WENGEL et al. 2008), but has recently been elevated to species level (REYNOLDS et al. 2014). As per its new definition, this species is restricted to the west of Madagascar where it has a patchy distribution mostly limited to habitats with trees and large shrubs. In Isalo, this species was found in both primary and secondary forests. In 2009, one young individual was observed resting for several days inside the Canyon of Zahavola, where it was probably hunting. None of the newly collected samples from Isalo could be successfully sequenced; we therefore here refer to a published record (16S, EU419803 – OROZCO-TER WENGEL et al. 2008).

**TYPHLOPIDAE**

*Madatyphlops arenarius* (GRANDIDIER, 1872)

Fig. 7L

Locality records: Ilakaka (FAZC 14696/MRSN R-uncatalogued), Ranohira (MRSN R2368–R2369).

*Madatyphlops arenarius* is a worm-like blind snake known from the west and south of Madagascar. It inhabits sandy substrates and can be encountered under stones and, rarely, active on the ground at night. In Isalo, this species is rarely found, and only a few records are currently available (three specimens have been collected). One specimen was found crossing a dirt road at night. Unfortunately, it was not possible to amplify DNA fragments from these recently collected specimens. The only genetic data available for this species from Isalo are those of a published record (COI, JQ909606; NAGY et al. 2012, 2015). This sample has 11% genetic distance to a sample of *Madatyphlops arenarius* from Ifaty (KT316431; NAGY et al. 2012). In fact, a recent publication indicated that *M. arenarius* might be a complex of at least four different taxa and a systematic revision of this group is therefore needed to assign this name to one or more populations (NAGY et al. 2015).
Discussion

In this study, we generated reference sequences for almost all amphibians and reptiles known from Isalo. These include 1) 16S sequences of 20 of the 21 amphibian taxa previously listed by Mercurio et al. (2008), and 2) 16S sequences of one new taxon (Mantidactylus sp. aff. multiplicatus Ca65 “Isalo”) and two new species distribution records (Mantella sp. aff. expectata “South” and Scaphiophryne sp. aff. calcarata Ca02); finally, we 3) provide reference sequences for 40 of the 47 reptile taxa currently known from this area. For seven taxa (Blaesodactylus sakalava, Geckolepis typica, Grandidierina rubrocaudata, Leioheterodon madagascariensis, Pseudoxyrhopus quinquelineatus, Tracheloptychus madagascariensis, and Zonosaurus ornatus) we only have reports from literature (Brygoo 1981, 1985, Hawkins 1994, Glaw & Vences 1994), as those taxa were not observed by our team during the multiple surveys we conducted in Isalo. In his species inventory from Isalo National Park, Hawkins (1994) reported on Lygodactylus tolampyae (Grandidier, 1872), a taxon already known from the Zombitse-Vohibasia National Park (Glaw & Vences 2007), and on L. gaimardi (Schlegel, 1837). Although we could not verify the taxonomic identities of these records we think they might refer to one of the two candidate species of Lygodactylus reported herein, and to L. guentheri or Lycodyras sp. aff. pseudogranuliceps Ca01 “Andohahela”, respectively.

Overall, when comparing our results with those available from other dry deciduous forests in Madagascar, it would appear that amphibian species richness (24 species-level taxa) in the Isalo Massif is relatively high and more diverse than at other western sites such as the Tsingy de Bemaraha National Park [9 species; Bora et al. (2010)], the Kirindy dry forest [15; Glos (2003)], Ankarakantsika National Park, and in the Montagne des Français [9; Mori et al. (2006) and D’Cruze et al. (2007)]. The scenario is slightly different for reptiles: compared to the 47 species-level taxa of reptiles in Isalo, there are higher numbers in Tsingy de Bemaraha (60, Bora et al. 2010), 58 in Mikea (Raselimanana 2008), 52 in the Montagne des Français (D’Cruze et al. 2007), whereas Kirindy and Ankarafantsika host similar numbers (43 and 47 taxa, respectively; Bloxam et al. 1996, Ramanamanjato & Rabibisoa 2002). If our results are compared with values available from the eastern rainforests, amphibian richness is obviously much lower (see for comparison Gehring et al. 2010, Rosa et al. 2012, Heinermann et al. 2015), while values for reptile richness are roughly similar (Andreone et al. 2003, Goodman et al. 2003, Kremen 2003, D’Cruze et al. 2008, Gehring et al. 2010).

The high level of species richness and the apparently locally endemic species observed in Isalo can be interpreted as a result of palaeoclimatic oscillations (Raxworthy & Nussbaum 1997, Mercier & Wilmé 2013). If this is true, the rainforest species could have expanded their ranges and survived in the massif during warm and humid periods due to habitat availability and the presence of permanent water and sub-humid forests, while dryness-adapted species remained constrained to small refugia in suitable areas (Crowley 2010, Mercier & Wilmé 2013); and the situation would have been the opposite during colder and drier periods. On the other hand, the peculiar position of the Isalo Massif and the structure of the sandstone formation that provides a number of humid (canyons) and arid habitats might have favoured the coexistence of taxa typical of multiple biomes. This heterogeneity allows for the presence of species adapted to different habitats, from the eastern humid forest, via the western dry deciduous forest to the southern subarid savannas, as is demonstrated by the presence of typical rainforest (e.g., Boophis luteus) and dry habitat (e.g., Brookesia brygooi) species. For some taxa, Isalo can also be characterized as a contact zone of different lineages such as those found in Trachylepis gravenhorstii and Madagascarophis meridionalis, and of closely related taxa such as Scaphiophryne sp. aff. calcarata Ca01 and Ca02. In the case of T. gravenhorstii, lineage 4a was already known from Isalo (Analalava Forest) as well as Anetoara, Ranomafana and Vohiparara (Vences et al. 2014). These latter three localities are all located on the humid eastern side of the island. Lineage 4b is instead widespread in the subarid-subhumid southern area (Tolagnaro, Andohahela, Manantaneley, Tranomaro, Ambolavao and Toliara; Vences et al. 2014). Similarly, the samples from Zahavola, Ranohira and Malaso of M. meridionalis correspond to the lineage known from Tsinanampetsotsa (arid environment) whereas the one from the Canyon des Rats, together with samples known from Ifaty and Toliara, belongs to the lineage present at Ihosy and Ranomafana (humid environment).

Figure 8. Photos of preserved specimens of Paroedura sp. aff. bastardi Ca01 “Marofandilia/Miandrivazo” (A; MRSN R2553) and Paroedura sp. aff. bastardi Ca03 “Ilakaka” (B; MRSN R2568).
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The analysis of populations of *M. expectata* (Figs 3A–C, J), M. sp. aff. *expectata* "South" (Figs 3D–E) and *M. betsileo* (Figs 3F–I) revealed that these taxa vary greatly in their colour patterns (Fig. 3). Interestingly, in Sakavato, two morphologically similar individuals with intermediate (*M. expectata/M. betsileo*) chromatic traits (Figs 3E, G) were identified by their mitochondrial DNA and their analysis revealed that these individuals represent two different taxa (M. sp. aff. *expectata* "South" – Fig. 3E, and *M. betsileo* – Fig. 3G). This might be due to introgressive hybridization of the mtDNA (CHIARI et al. 2004, COTTINI et al. 2008) or convergent evolution of the same coloration pattern promoted by living in the same environment and sharing the same selective pressures, for example predation (Müllerian mimicry; CHIARI et al. 2004).

In this study, we provide a thorough taxonomic identification of most of the Isalo specimens hosted in the MRSN collection (Supplementary Table S3 and S5) and found that only *Scaphiophyrene menabensis* is not represented in its amphibian collection. As far as reptiles are concerned, *Crocodylus niloticus*, *Lygodactylus* sp. aff. *Richtersia* "Isalo", *Blaesodactylus sakalava*, *Geckolespis typica*, *Paroedura* sp. aff. *bardi* Ca02 "Isalo", *Phelsuma mutabilis*, *Phelsuma standingi*, *Zonosaurus karsteni*, *Zonosaurus ornatus*, *Tracheloptychus madagascariensis*, *Grandidierina rubrocaudata*, *Thamnophis lateralis*, *Sanzinia voluntary*, *Acrantophis dunnerli*, *Leioheterodon madagascariensis*, and *Pseudoxyrhopus quinquelineatus* are currently not represented in the MRSN collection. The findings reported in this study highlight the crucial importance of combining extensive field research with molecular taxonomic identification and with the morphological inspection of voucher specimens hosted in museum collections (ROCHA et al. 2014). With this approach, it is possible to generate taxonomically accurate species lists and (when necessary) update previously published work to accurately assess the biodiversity value of a surveyed area. In some instances, the lack of reference sequences in public repositories did not allow a sample to be unequivocally assigned to a described or undescribed taxa. In these cases, their identification was facilitated only by the availability of corresponding museum specimens (e.g., *Paroedura vahiny* and *Lygodryas guentheri*). At the same time the availability of preserved specimens enabled us to ascertain the consistency of diagnostic characters. This was the case in our *Leioheterodon* samples that we assigned to *L. modestus* on the basis of diagnostic morphological traits when molecular data were misleading. It provided a possibility to extract new tissue samples from existing specimens that facilitated the sequencing and reassembly of one *G. azzurrae* paratype (MRSN A5313) to *G. corvus* and the investigation of other aspects of specimen morphology (e.g., coloration, as in some specimens of *Mantella* spp. analysed in our study). All this underscores the crucial role that historical and modern collections play in cataloguing world biodiversity and the importance of maintaining specimens in permanent and accessible museum collections (DE LA SANCHE et al. 2016, ANDREONE 2017).

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References


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Supplementary material

8 Supplementary Tables:
Table S1. List of toponyms and corresponding GPS coordinates.
Table S2. List of amphibian samples analysed for this study.
Table S3. List of Isalo's voucher specimens of amphibians unequivocally identified based on morphology and/or molecular analyses.
Table S4. List of reptile samples analysed for this study.
Table S5. List of Isalo's voucher specimens of reptiles unequivocally identified based on morphology and/or molecular analyses.
Table S6. Primer sequences and PCR conditions used in the present study.
Table S7. Pairwise genetic distances (p-distances) between and within amphibian taxa according to 16S rRNA gene fragment sequence variation.
Table S8. Pairwise genetic distances (p-distance) between and within reptile taxa according to COI gene fragment sequence variation.