Light as an "ecological trap". A synanthropic incipient speciation taken up by the gecko

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Running title: Anthropogenic speciation by light

Abstract

Two distinct and sympatric populations of Moorish gecko (*Tarentola mauritanica*) can be easily distinguished: the diurnal-dark gecko, which mainly lives on trees, and the nocturnal-pale gecko, recognizable on anthropic buildings' walls, especially nearby artificial lights, in order to increase the chances to get preys.

Here we hypothesize that this evolutionary splitting was determined by the choice of the anthropogenic niche occurring in a population of Moorish gecko back in the time when *Homo sapiens* used fire in its settlements, luring insects to positive phototaxis. The frequentation of the new trophic niche created by human through light has increasingly strengthened the distinctive traits in the nocturnal population of geckos, driving sympatric speciation by selection for background matching.

We provide empirical evidence supporting this hypothesis, suggesting that the nocturnal-pale geckos split from the diurnal-dark populations due to the innate plastic variation determined by their ability to adjust their skin color and later reinforcement of depigmentation.

Our study inaugurates an interesting line of investigation that could shed light on the impact that humankind has on the evolutionary trajectories of non-domesticated species, endowed with adaptive plasticity, functional to the exploration and colonization of the anthropic niche.

Keywords

Homo sapiens, Fire Era, Moorish gecko, synanthropism

Introduction

The history of *Homo sapiens* is studded with many events which promoted relationships with wild animals in different ways (Ålund et al. 2023; Hulme-Beaman et al. 2016), changing their evolutionary path or, somehow, impacting their adaptation (Van Bocxlaer et al. 2010; Puckett et al. 2020).

Whereas these “domestication” events are generally recognized as the product of planned actions, aimed to annex useful plant and animal species into the anthropic niche, some organisms established synanthropic relationships beyond the human intention. These latter occurrences are noticeable in many evolutionary processes as the self-domestication developed in mice and rats (Pocock et al. 2004), sparrows (Ravinet et al. 2018), cockroaches (Booth et al. 2011), foxes (Parsons et al. 2020) , and elephants (Raviv et al. 2023).

Humans' connotation as “niche builders” (Kendal et al. 2011), referring to the unintentional exploitation of other species, is not limited to the management of food, but it's also conceivable as an “ecological trap” (Schlaepfer et al. 2002; Hale and Swearer 2016).

Animals may find opportunities to make dens, shelters, and microclimatic buffering (Kamp et al. 2009) within human environments. Artificial light is present mostly in urban agglomeration, but it irremediably spreads to natural ecosystems, this could lead to adaptive changes that influence temporal niches and trophic interactions in different groups of animals (Jägerbrand & Spoelstra, 2023). Several studies documented the effect of artificial light at night on animals (Wang et al. 2021; Baker and Richardson 2006; Beier 2006; Russo et al. 2017), including Moorish gecko, *Tarentola mauritanica* (Martín et al. 2018), mainly focusing on physiological and behavioral conditioning.

Herein, we report data corroborating the hypothesis of an evolutionary splitting determined by synanthropism, occurring in the Moorish gecko, dating back to the time when *Homo sapiens* used fire in its settlements.

The Moorish gecko is a small saurian widespread in North Africa and Europe. This species has the widest range in the Mediterranean Basin compared to all geckos' species (Rieppel 1981). However, because of the close relationship with humans, the gecko has been accidentally introduced in the Balearics (Spain), Madeira island (Portugal), some Balkan islands, Crete, and South America (Carranza et al. 2000; IUCN 2017) (Fig. 1). European populations of Moorish gecko diverged during the early Pliocene, around 4.14 Mya. In fact, the colonization of the Iberian Peninsula by Moorish gecko from North Africa could have been during the Messinian Salinity Crisis, or after the opening of the Strait of Gibraltar during the early Pliocene (Rato et al. 2012).

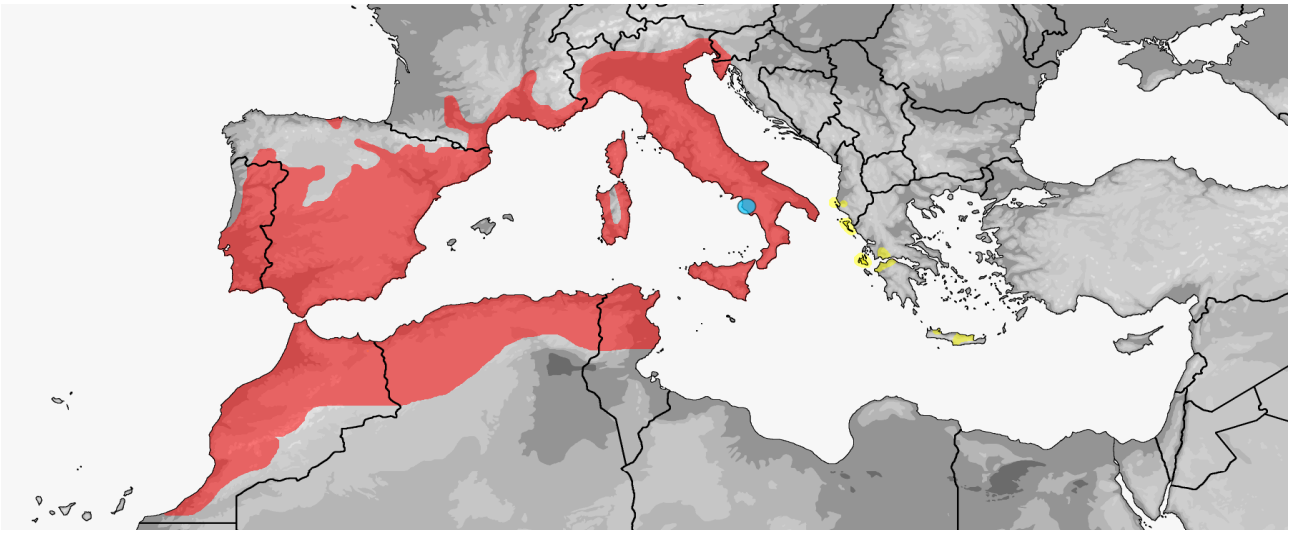


Figure 1 – Moorish gecko distribution range. Native (red) and introduction (yellow) range distribution of Moorish gecko (*Tarentola mauritanica*). The map was by © Rune Midtgaard, "RepFocus - A survey of Reptiles of the World" (https://repfocus.dk). The blue spot is referred to the study site.

The Moorish gecko is active during diurnal and nocturnal hours, occupying a variety of ecological niches, such as tree trunks, houses, and stone walls (Fulgione et al. 2019; Rato et al. 2023; Valverde

2004). This species represents an interesting example of plastic variation, highlighted by the ability to accommodate its skin color according to the substrate (Vroonen et al. 2012) (Fig. 2).

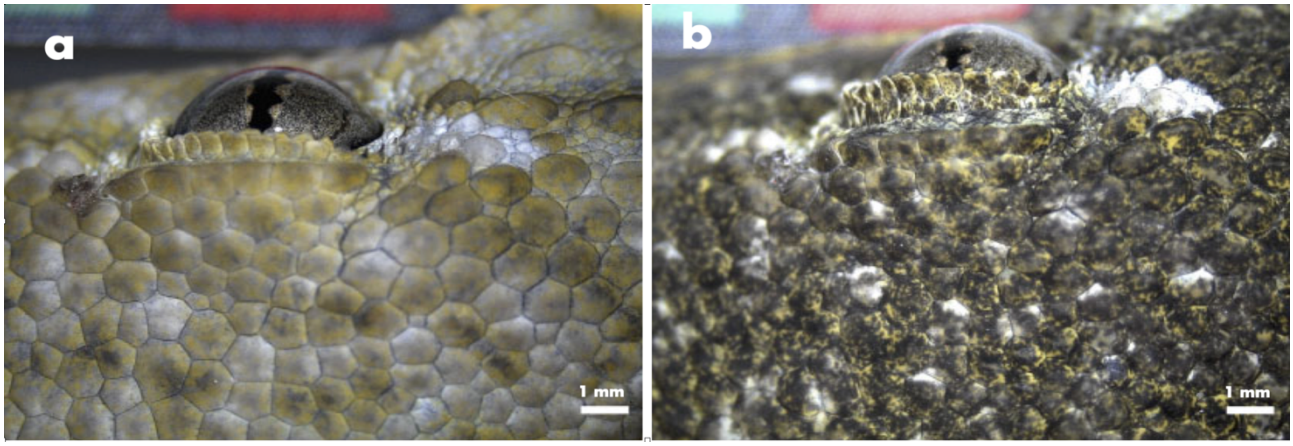


Figure 2 – **Skin color change**. Variation in skin colouration of Moorish gecko placed on light (a) and dark (b) substrate. The color change occurred in 1.5 hours.

Two distinct and sympatric populations can be easily distinguished (Fulgione et al. 2019): the -dark diurnal-gecko which mainly lives on trees, and the pale-nocturnal gecko, recognizable on anthropic buildings' walls, especially nearby artificial lights, in order to increase the chances to get preys (Fulgione et al. 2019; Arnold 2002; Rato and Carretero 2015) (Fig. 3).

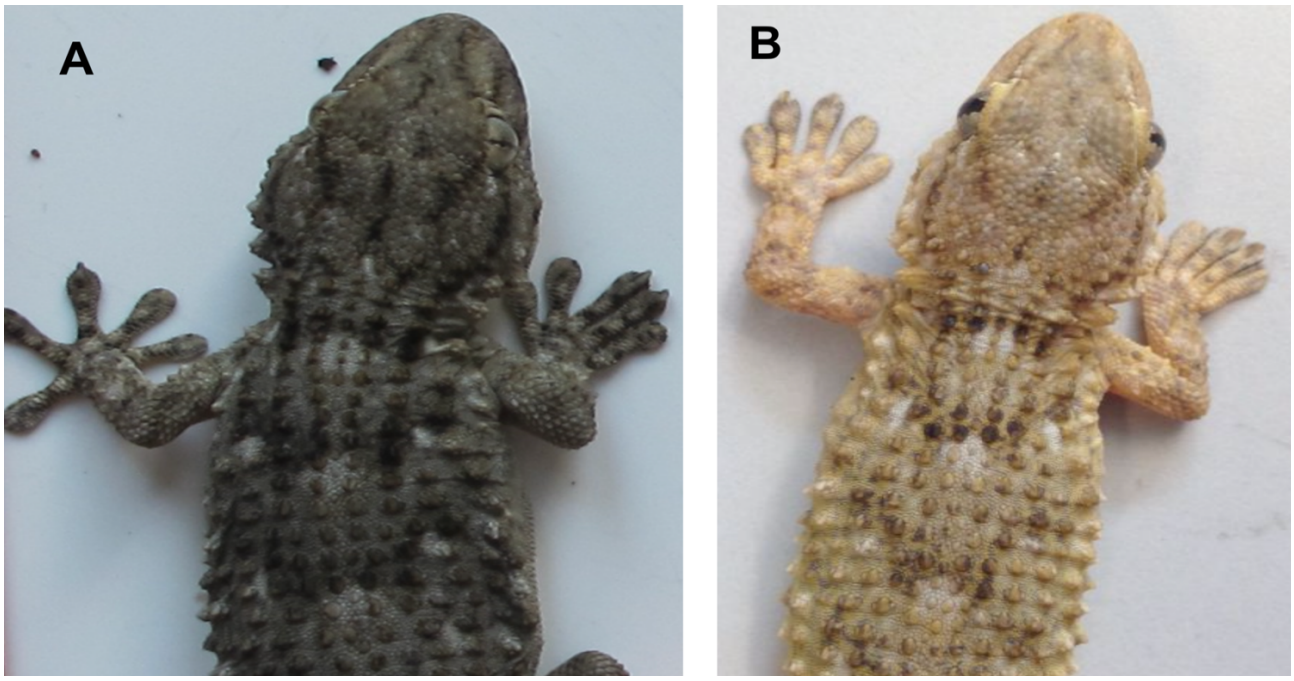


Figure 3 – **Geckos' morphotypes**. Dark-diurnal (A) and pale-nocturnal (B) Moorish geckos (*Tarentola mauritanica*). They occupy two distinct ecological niches: logs during the day and the walls of houses at night, respectively.

We hypothesized that the pale gecko, active during the night, may have experienced a prolonged evolution alongside humans, rooted in the deep past, when *H. sapiens* populations occupied the first caves and used fire. Since then, the nocturnal gecko has accompanied our species and the evolution of housing structures.

We provide empirical evidence supporting this hypothesis, suggesting that the nocturnal-pale geckos split from the diurnal-dark populations due to the innate plastic variation determined by their ability to adjust their skin color (Vroonen et al. 2012; Fulgione et al. 2019, 2014) and later reinforcement of depigmentation. This attitude could have been useful when humans added a new niche dimension to the natural environment, lighting up fires on purpose to get illumination and heating.

Humans have been using fire for hundreds of thousands of years: the intentional use of fire traces back to the Middle Pleistocene of the Levant (Zohar et al. 2022; Stepka et al. 2022) but understandably spread as a common practice just afterward (Shimelmitz et al. 2014).

Whereas earliest fire usage may suggest cooking and anti-predatory activities, the *daylight extension*, typical of the human social niche, may have appeared later, and could have been linked to the development of language during the latest Pleistocene (Gowlett 2016).

The *daylight extension* hypothesis is consistent with the observation that humans have developed an activity peak during late evening hours, an unusual condition comparing to the mammalian standard (Bowman et al. 2011).

This phenomenon may have unintentionally provided the Moorish gecko with abundant insect preys, especially among insects species characterized by positive phototaxis (i.e. moths, grasshoppers, and flies) (Park and Lee 2017).

This extraordinary new food bonanza could be exploited only using crypticism, a specialization that prevents the geckos from being preys in their turn of nocturnal predators, which is led by their skin-color variation ability (Vroonen et al. 2012). Moreover, geckos with adhesive toepads can easily scale vertical or even inverted surfaces (Gamble et al. 2012), and these extraordinary clinging abilities may have facilitated the use of smooth walls inside the caves.

To test this hypothesis, we have studied two sympatric populations of geckos, a dark-diurnal and a pale-nocturnal one. First, we investigated the two populations' phylogenetic history to date their split age. Then, we produced habitat suitability models for the gecko, and we tested them for a positive association with the human fossil sites dating back to the split age. Molecular dating of the phyletic separation between diurnal and nocturnal geckos, together with the spatial correspondence between geckos and human populations, could prove the beginning of the phenomenon. Moreover, a palaeoecological experiment was performed to demonstrate the effective attraction of Arthropods

130 on wall lighted with fire. The subsequent reinforcement, through the depigmentation effect
131 observed in pale-nocturnal geckos, was shown using histology of melanosomes and quantified the
132 markers of melanization.
133
134 **Materials and Methods**
135 ***Sampling and study area***
136 The study was carried out in South Italy (40°15'N, 14°54'E, Salerno province) (Figure. 1). The area
137 was dominated by Mediterranean vegetation, characterized by olive tree cultivation, rural buildings,
138 and stone walls.
139 By using nylon loops, we sampled 16 individuals: 6 dark-diurnal geckos collected on the trunks, and
140 10 pale-nocturnal geckos captured on the wall.
141 For each gecko, a tip of the tail was collected after its autotomy and preserved in a sterile tube with
142 70% Et-OH, stored at -20°C until the processing in the laboratory. After tissue collection, the animals
143 were released at the point of capture, except for 6 individuals (3 dark-diurnal geckos and 3 pale-
144 nocturnal geckos) that were sacrificed for sampling tissues useful for histological analyses (see after).
145 The animals were sampled with the permission of the local county authority (Cilento, Vallo di Diano
146 e Alburni National Park, prot. 2013/0010678). All experimental protocols were approved by the
147 Ethical Committee for Animal Experiments University of Naples Federico II (protocol 2013/0032826).
148
149 **Mitochondrial genomic characterization**
150 ***DNA extraction and sequencing***
151 Total mitochondrial DNA (mtDNA) was extracted from 25 mg of tissue using miniprep PureLink
152 HiPure Plasmid DNA Purification Kits (Invitrogen), according to manufacturer's recommendations,
153 except for initial mechanical disruption of samples: the procedure followed is 1h in a Phosphate

154 Buffer Saline (PBS). Blank extractions were performed at each extraction to monitor potential cross-
155 contaminations. Finally, we checked the concentration and quality of DNA using the Qubit
156 Fluorometer (Thermofisher Scientific) and the Agilent 4200 TapeStation system (Agilent
157 Technologies), respectively.
158 High throughput sequencing of the mitochondrial genomes was performed on the Illumina 2500
159 platform using the Nextera XT DNA Sample Preparation protocol at the Genomix4Life Srl
160 (http://www.genomix4life.com/ it/).
161 Processing of raw sequencing data was performed as reported in (Fulgione et al. 2019). In brief, the
162 FastQC software v0.11.4 (http://www.bioinformatics. babraham.ac.uk) was used to quality control
163 check of raw sequence data. The Trimmomatic software v0.35 (Bolger et al. 2014), was
164 implemented, in paired end mode, to trim and crop the low-quality raw reads (Q < 28), and to
165 remove the primers and Illumina adapters. Paired reads were processed simultaneously, while
166 orphan reads were discarded from the subsequent analyses.
167 The assembly was performed in Geneious version R9.16 (Kearse et al. 2012) in mapping assembler
168 mode. For each sample, the forward and reverse filtered reads were used to create contiguous
169 sequences (contigs) in *de novo* assembly procedure. To check the quality of assembly, the contig
170 sequences have been mapped against a *T. mauritanica* reference sequence (NCBI accession number
171 EU443255).
172
173 ***Phylogenetic characterization and time tree***
174 To evaluate the haplotype of sequenced mitochondrial genomes, we aligned them in Geneious
175 version R9.16 with sequences of *T. mauritanica* (NCBI accession number from JQ300539 to
176 JQ301443), representative of different lineages (Rato et al. 2012), using *Gekko gecko* (accession
177 number AY282753) as the outgroup.

178 To infer the split time of pale-nocturnal and dark-diurnal geckos' populations, we elaborated a time-
179 tree in MEGA11 (Tamura et al. 2021). We used 10 pale-nocturnal, 6 dark-diurnal geckos sequences,
180 and *T. mauritanica* sequence belonging to Clade I (accession number JQ425050) as outgroup. We
181 also added a *T. mauritanica* Clade II sequence (accession number JQ425041) to allow a better
182 resolution of time-lines. Indeed, Clade I and II are temporally the closest (Rato et al. 2012) .
183 Before that, to evaluate the haplotype for JQ425041 and JQ425050, we aligned these with
184 sequences of *T.mauritanica* from JQ300539 to JQ301443 (Rato et al. 2012), representative of
185 different lineages of *T. mauritanica*.
186 Firstly, an alignment with Muscle was performed considering the 18 mtDNA sequences. The
187 following parameters were set: gapopn = -400.00, gapextend = 0.00, max iters = 16, 2 iterations,
188 cluster methods = UPGMA.
189 Maximum-Likelihood uses several substitution models to correct for multiple changes at the same
190 site during the evolutionary history of the sequences. To set the best model, we perform a test
191 considering the lowest Bayesian Information Criteria (BIC). According this criteria the Tamura-Nei
192 method was selected (BIC:52037,98) for the construction of the phylogenetic tree.
193 A timetree was inferred by applying the RelTime method (Tamura et al. 2012, 2018) on 18TN93Tree
194 in MEGA11 (Tamura et al. 2021) using a dataset of 16,618 positions.
195 Branch lengths were calculated using the Maximum Likelihood (ML) method and the General Time
196 Reversible substitution model (Nei and Kumar 2000).
197 The timetree was computed using 1 calibration constraint. Times were not estimated for outgroup
198 nodes, because the Reltime method uses evolutionary rates from the ingroup to calculate
199 divergence times and doesn't assume that evolutionary rates in the ingroup clade apply to the
200 outgroup.

The estimated log likelihood value of the tree is -25,781.91. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G; parameter = 0.6794).

To confirm the results obtained with MEGA11, the divergence time estimation was performed also with BEAST v1.14 (Suchard et al. 2018), setting the following parameters: Substitution Model= TN93, Gamma Category Count= 5, Strick Clock Model and setting priors to Coalescent Constant Population.

Species Distribution models

For both species, we defined the Mediterranean region as the study area, according to the current and historical (Rato et al. 2012) geographical distribution of *T. mauritanica*.

Then, we generated by random sampling 10000 background points within the background area.

To account for potential sampling biases, pseudoabsences were geographically placed according to the density of the occurrence data, so pseudoabsences are more abundant where presences are denser (Phillips and Dudík 2008; Syfert et al. 2013; Roy-Dufresne et al. 2019).

For *H. sapiens* only, the record was divided into 1000-years consecutive time bins according to the time resolution of bioclimatic predictors.

Subsequently, we partitioned the pseudoabsences proportionally to the number of presences per time bin.

After extracting climatic values in each occurrence and pseudoabsence datapoints, we accounted for multicollinearity among predictors by considering the Variance Inflation Factor (VIF).

The latter was assessed by using the function “vifcor” embedded in the “usdm” R package and selecting a threshold of 0.75 (Zuur et al. 2010; Naimi et al. 2014).

After VIF application, the selected predictors were BIO4 (temperature seasonality), BIO8 (mean temperature of the wettest quarter), BIO 9 (mean temperature of driest quarter), BIO 13

224 (Precipitation of Wettest Month), BIO 14 (precipitation of driest month), BIO 15 (Precipitation
225 Seasonality), BIO 19 (precipitation of coldest quarter).
226 To estimate the geographic distribution of both species in the past, we adopted the SDM ensemble
227 approach. Specifically, we trained SDMs through an ensemble forecasting approach, as implemented
228 in the R package "biomod2" (Thuiller et al. 2009) .
229 We considered four different algorithms: Maximum Entropy Models (MaxEnt), Generalized Boosted
230 Models (GBM), Random Forests (RF), and Generalized Linear Models (GLM). As model tuning, we
231 adopted the default setting described in "biomod" R package. To evaluate the predictive accuracy of
232 SDMs, we randomly split each occurrence dataset into a 70% sample, used for the calibration of the
233 model, and the remaining 30%, used to evaluate model performance. Then, we calculated the area
234 under the receiver operating characteristic curve (AUC (Hanley and McNeil 1982)) and the true skill
235 statistic (TSS (Allouche et al. 2006)). This entire procedure was iterated 10 times, changing the
236 randomly selected training/testing datapoints at each iteration. Model averaging was performed by
237 weighting the individual model projection by their AUC values and averaging the results (Marmion
238 et al. 2009) after excluding the model with AUC <0.7.
239 Lastly, SDM predictions were projected in the past to obtain spatial explicit habitat suitability of *H.*
240 *sapiens* and *T. mauritanica* in the Mediterranean area during the 60-20 kyr interval.
241
242 **Human-gecko sympatry**
243 We collected modern occurrence data for *T. mauritanica* from the "Global Biodiversity Information
244 Facility" (GBIF, www.gbif.org/) database by selecting "human observation" and "machine
245 observation" as the basis of the record.
246 The data were further filtered by removing duplicate records and occurrence records devoid of
247 geographical coordinates.

For the human dataset, we implemented the *H. sapiens* record published in (Raia et al. 2020) and (Timmermann et al. 2022), by focusing on the fossil presence observations dated in between 60 and 20 kya.

Radiocarbon data were calibrated by using the "Bchron" R package (Haslett and Parnell 2008) through the "intcal20" curve (Reimer et al. 2020).

As environmental predictors, we used the monthly bioclimatic variables generated through the 2Ma CESM1.2 simulation (Timmermann et al. 2022) downscaled at 0.5° × 0.5° grid resolution. The native set of predictors was subsequently converted in the bioclimatic variables according to WorldClim using the "dismo" R package (Hijmans et al. 2023). Lastly, variables were projected on the Mollweide coordinate reference system.

To prevent model over-fitting, duplicate occurrences in raster grid were removed. After this step, we gathered on 520 modern *T. mauritanica* and 740 past *H. sapiens* occurrences.

***Homo sapiens* – *Tarentola mauritanica* spatial correlation**

To verify whether there was a significant spatial correlation between *H. sapiens* and *T. mauritanica*, we adopted the Boyce Index (Hirzel et al. 2006). It requires presences datapoints only and measures how much model predictions differ from a random distribution of the observed presences across the prediction gradients (Boyce et al. 2002). Boyce Index varies between -1 and +1, whereby positive values indicate a model which present predictions are consistent with the distribution of presences in the evaluation dataset, values close to zero mean that the model is not different from a random model, negative values indicate counter predictions, i.e., predicting poor quality areas where presences are more frequent (Hirzel et al. 2006).

In our case, we used Boyce Index to compare the prediction of *T. mauritanica* in the past associated to human presence and the prediction of *T. mauritanica* across the entire background area (i.e., Mediterranean area).

Moreover, we trained a Linear Mixed-Effects Model to test for the presence of ecological correlation between humans and the Moorish gecko in the past. To this aim, we merged the habitat suitability values of the two species associated with both human presence and pseudoabsence datapoints across time. Lined Models were fitted by selecting the habitat suitability values as independent and response variables and by choosing the "time" as a random effect.

Paleoecology simulation

Two walls of 4x4 square meters each were set up on a site within the study area. A wall has a fire placed at the base and the other was without.

Over the course of 5 hours at night, all the Arthropoda resting on the two walls were captured and identified through the examination of diagnostic morphological traits under a stereomicroscope Leica EZ4 W and specialized reference manual (Bellmann 2019). We recorded the Orders and the corresponding number of individuals and then determine diversity and richness by Past3 software (Hammer et al. 2001).

Reflectance

To objectively determine the skin coloration of sampled geckos, we measured the skin reflectance by spectrophotometry (250–1000 nm, AvaSpec-2048-USB2-UA-50; Avantes, Apeldorn, Netherlands) considered a range between 300 and 700 nm (Vroonen et al. 2012). A white reference tile (WS2; Avantes) was used as a reference for calibration.

293 The spectrophotometer probe (with a hole end of 0.2 mm) was held perpendicular to the body
294 surface of animals and the reflectance (R%) was recorded in three positions on the back. Then, the
295 average of the integrals subtended the reflectance curves was assumed to be representative of the
296 whole back of each individual (Vroonen et al. 2012; Fulgione et al. 2019, 2015). The difference
297 between diurnal/dark and nocturnal/pale groups was assessed by a one-way ANOVA test. And
298 statistical significance was defined as $p < 0.05$.
299
300 **Skin histology**
301 Three dark and three light geckos were anaesthetized with 250 mg/kg 1% MS-222 (Sigma Chemical
302 Co. St. Luis, MO) injected into the intracoelomic cavity and were sacrificed by decapitation. Skin
303 samples deriving from 3 dark and 3 light geckos' backs, were processed for light microscopy as in
304 (Avallone et al. 2018). Briefly, after fixation in 2% paraformaldehyde and 2.5% glutaraldehyde (4h at
305 4 °C) and post-fixation in 2% osmium tetroxide solution (1 h, at 4 °C), they were dehydrated in
306 ascending series of ethyl alcohol and embedded in Epon 812 resin (Fluka). Semi-thin sections of 1.5
307 µm were cut with a Super Nova Leica Ultratome and stained with 1% toluidine blue in 1% sodium
308 tetraborate buffer. Sections were examined with a Zeiss Axiocam camera applied to a Zeiss Axioskop
309 microscope (Zeiss, Jena, Germany).
310
311 **Melanocyte stimulating Hormone (α-MSH) and Melanin assay**
312 Plasma and skin were collected from 3 nocturnal/pale and 3 diurnal/dark geckos.
313 Blood was sampled with a heparinized syringe from interdiscal vertebra windows of the tail. The
314 plasma was isolated by centrifugation at 2000 g for 10 min at room temperature and samples were
315 stored at −80 °C until processing in laboratory.

α-MSH levels were determined by the ELISA assay as described in Monti and co-authors (Monti et al. 2013). The Student's t test was used to test statistical significance difference ($p < 0.05$) between the two groups.

To quantify melanin, we used protocol proposed by Kalie and co-authors (Kalie et al. 2013), with the following excepts: tissues collection in 50 mM Tris-HCl pH 7.4, 300 mM NaCl, 0.5% NP-40 and protease inhibitors (Roche), and lysing of cells by freeze-thawing (3 cycles of dried ice at -37 °C), Dounce homogenization (200 strokes) and sonication (2 min, 10 sec on and 10sec off).

Results

Divergence timing

The phylogeny of the considered geckos' populations, using the complete mitochondrial genome, highlights their monophyly by placing the common ancestor at about 500 thousand years ago (0.28-0.71 Mya) (Fig. 4). Subsequently, the topology shows a dichotomous division in two clades, dark-diurnal and pale-nocturnal, also monophyletic. The common ancestor of the pale-nocturnal geckos seems to be traced back to 40 thousand years ago (range 0.02 – 0.06 Mya).

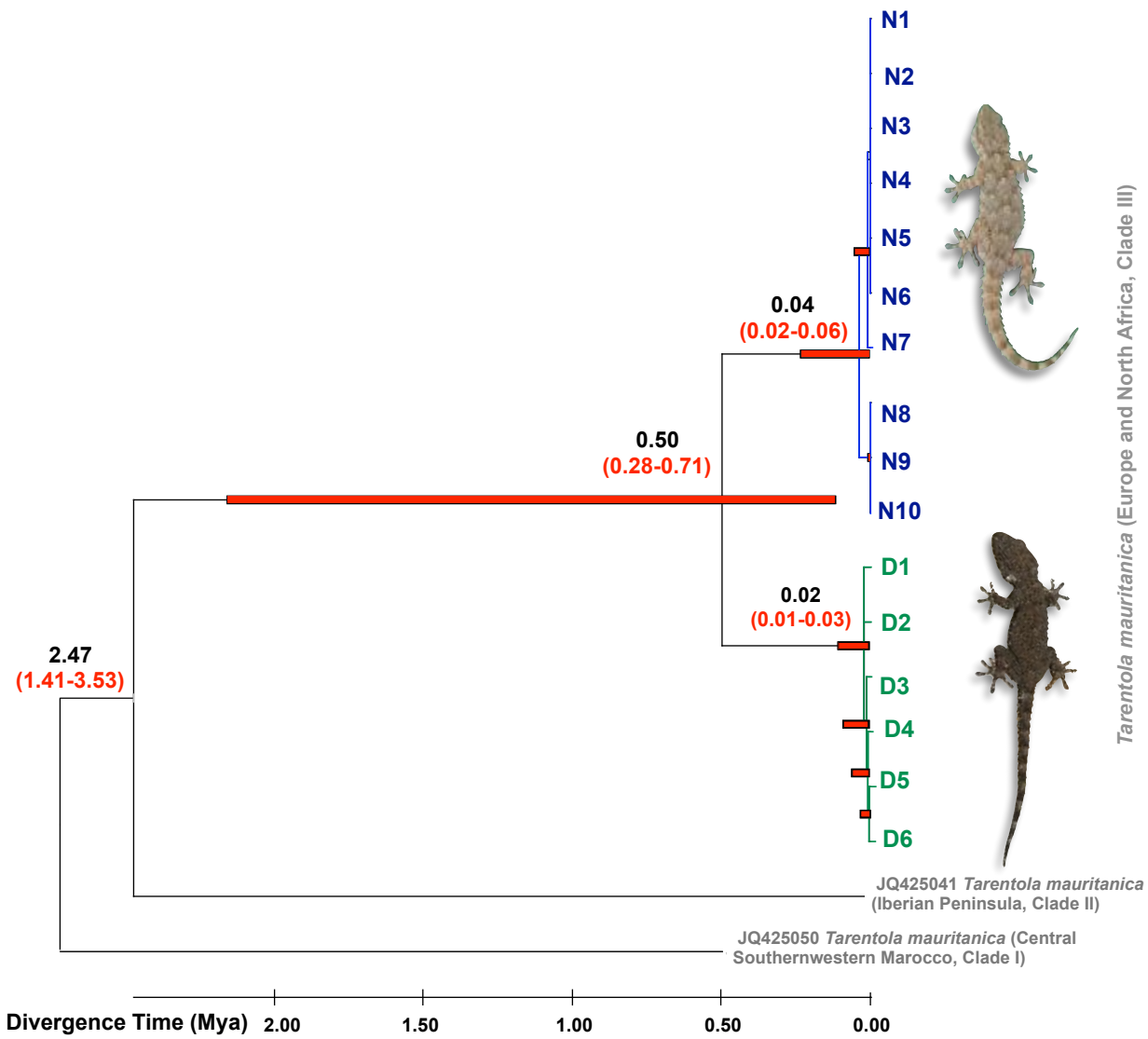


Figure 4 – Phylogenetic reconstruction. Phylogeny and divergence time estimation obtained by molecular-clock analysis of 10 pale-nocturnal (N1-N10) and 6 dark-diurnal geckos (D1-D6). Divergence times of the two gecko's populations were calculated using the complete mitochondrial genome. The red bar on the nodes indicates the 95% credibility intervals of the estimated posterior distributions of the divergence times. Mya: million years ago.

Species Distribution Models

The SDMs developed for humans reached high predictive performances, expressed by the AUC value equal to 0.818 (sd=0.014) and TSS equal to 0.502 (sd=0.028).

The SDMs developed for *T. mauritanica* reached very high performances, expressed by the AUC value equal to 0.96 (sd=0.002) and the TSS equal to 0.803 (sd=0.008).

We estimated the magnitude of the spatial correlation between humans and the Moorish gecko in the past using the Boyce Index. Specifically, we obtained a Spearman correlation value equal to 0.422.

Linear Mixed-Effects Model shows a positive and significant relationship between *H. sapiens* and *T. mauritanica* habitat suitability values ($p<0.001$) (Fig. 5). Furthermore, the model showed a relevant age-driven random effect structure ($p=0.016$).

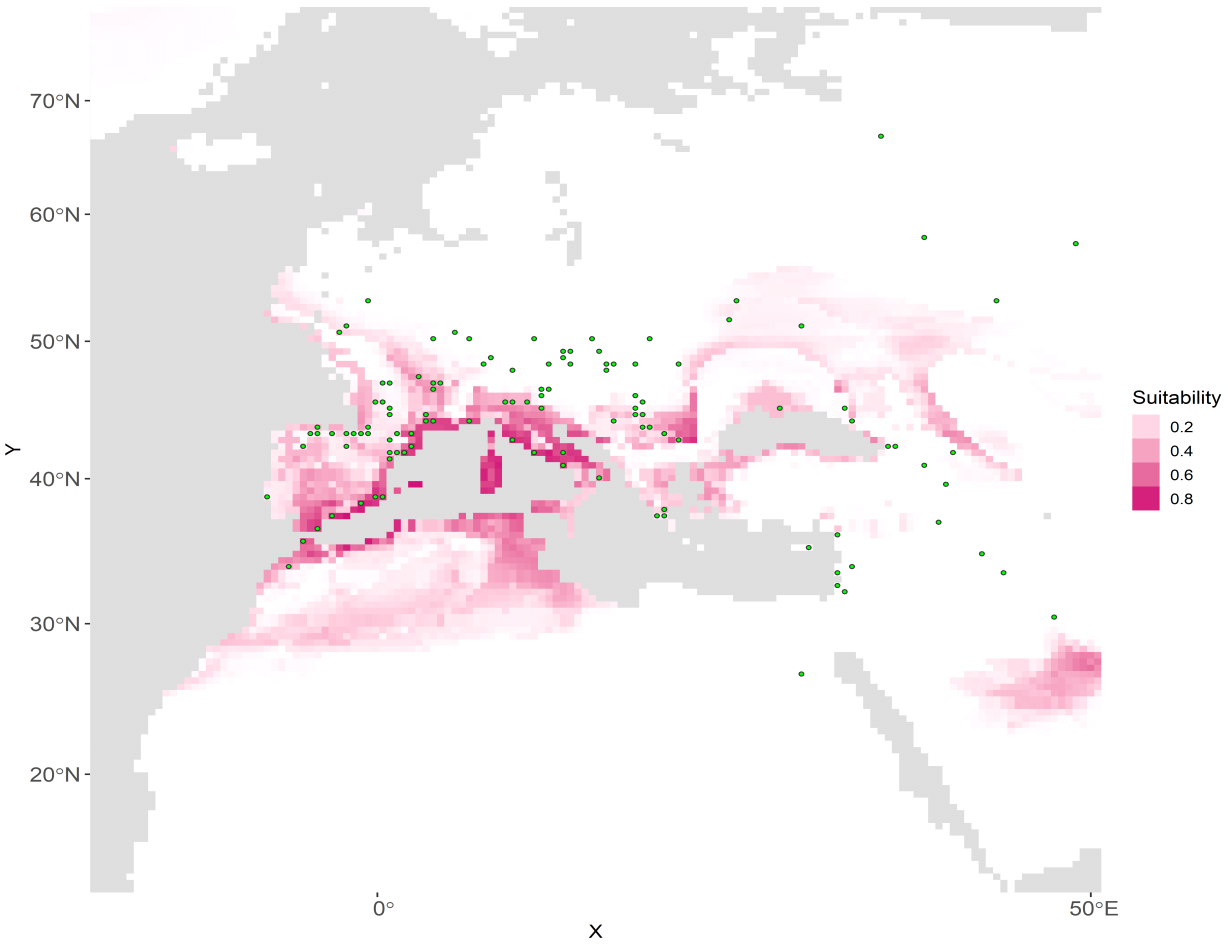
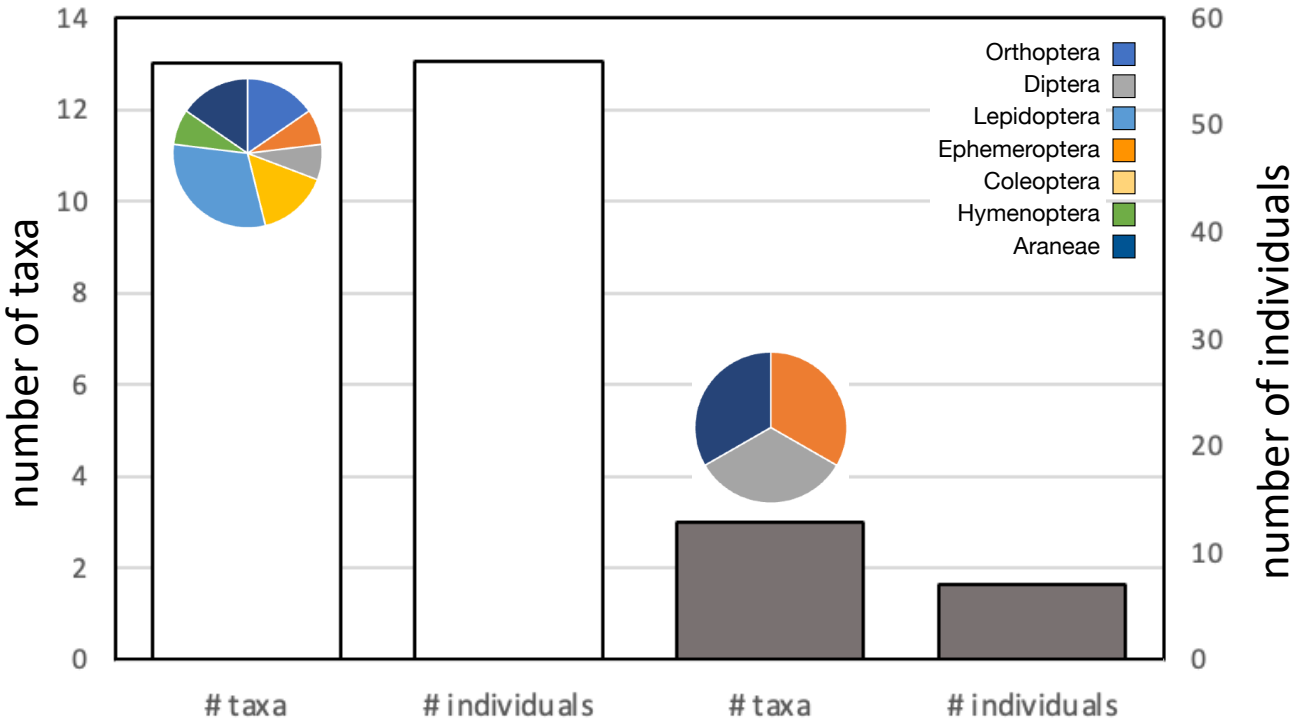


Figure 5 - Spatial distribution model of *Tarentola mauritanica* and *Homo sapiens* 40 kylo years age. Linear Mixed-Effects Model shows a positive and significant relationship between *H. sapiens* (green dots) and *T. mauritanica* habitat suitability (red area).

356 **Paleoecology simulation**

357 The paleoecology simulation experiment in the field made possible to determine the effectiveness
358 of a light source (fire) placed at the base of the wall in attracting Arthropods. The number and
359 diversity of taxa sampled to the wall lighted by fire was significantly higher than the wall without
360 input of fire (Fig. 6) (for diversity: Chi squared = 6.250 with 1 degrees of freedom; the two-tailed P =
361 0.0124. For abundance: Chi squared = 38.111 with 1 degrees of freedom; the two-tailed $P < 0.0001$).



362

363 **Figure 6 – Paleoecology simulation.** Arthropods richness and number of individuals on the walls
364 with (white histogram) and without (grey histogram) a light source. The colored pies represent the
365 proportion of taxa for each Order.

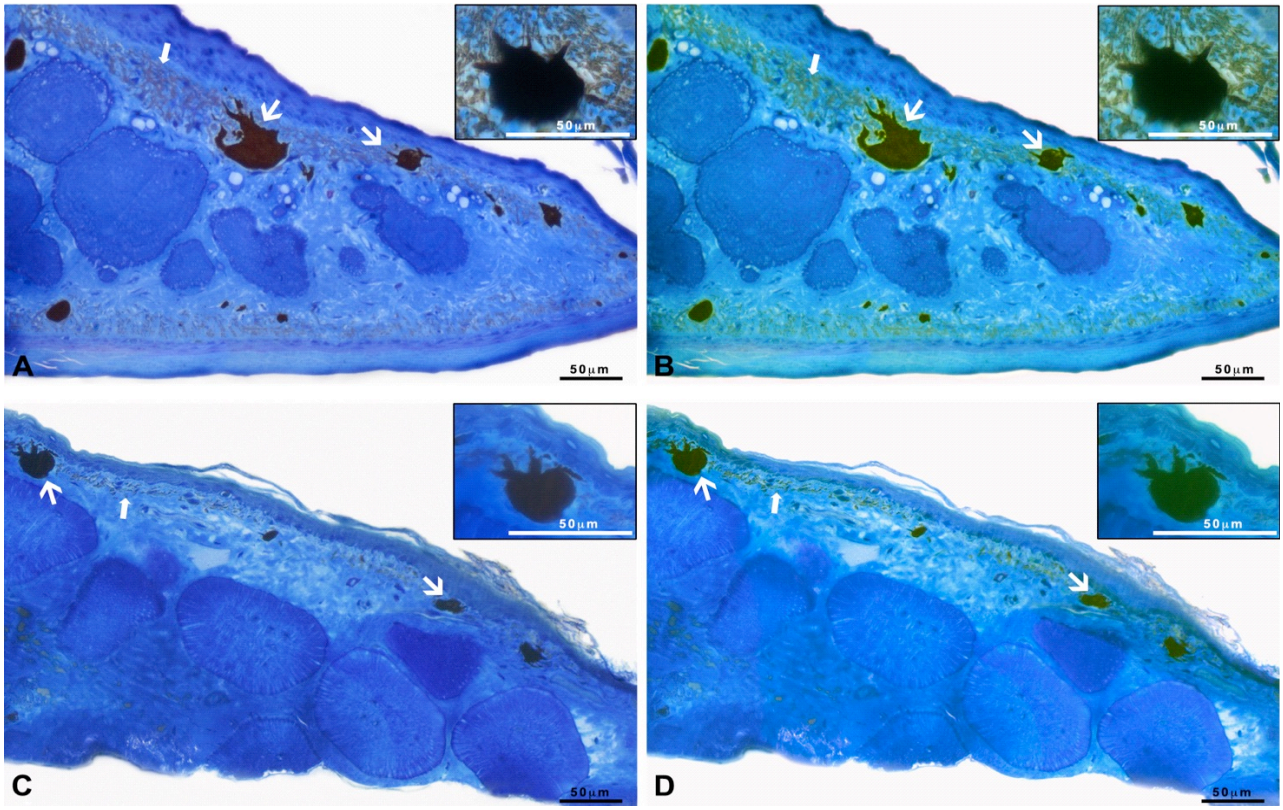
366

367 **Histological skin analysis**

368 Although the two populations (diurnal and nocturnal) retain the ability to vary the level of
369 darkness/lightness (Fulgione et al. 2019), nocturnal geckos are characterized by a marked
370 depigmentation. The comparison between histological skin samples taken from the backs of pale-

371 nocturnal and dark-diurnal geckos showed that dark phenotype geckos' skin has numerous,
372 predominantly large, melanophores. In addition, a thick and continuous layer of pigment granules is
373 observed in the connective tissue of the underlying dermis. By contrast, in the skin of pale-nocturnal
374 specimens, the melanophores appear remarkably reduced in size and less abundant, and the layer
375 of pigment surrounding them is exiguous (Fig. 7).

376



377

378 **Figure 7** - **Histological skin analysis.** Skin sections stained with toluidine blue. A) Skin from the backs
379 of dark-diurnal gecko, and single melanophores (inset). There is a high concentration of
380 melanophores (thick arrow) localized in the dermal region and a thick layer of melanin granule
381 (arrow). B) Picture A digitally stained to emphasize melanophores and melanin pigment. C) Skin from
382 the backs of a pale-nocturnal gecko and a single melanophore (inset). D) Picture C digitally stained
383 to emphasize melanophores and a layer of melanin pigment.

384

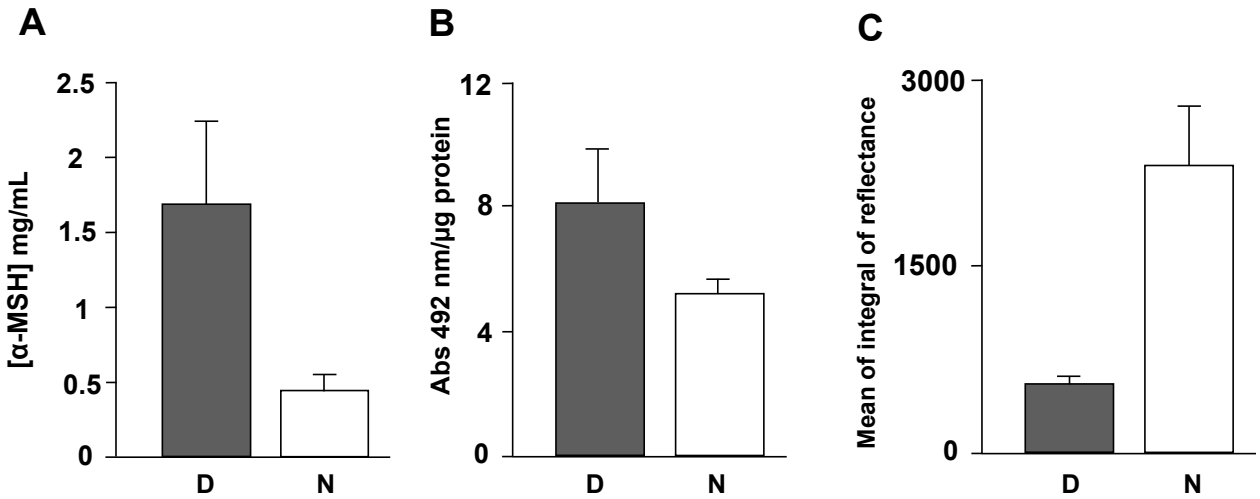
385

386 **α-MSH and Melanin assay**

387 The α-MSH hormone, produced by the pituitary gland, stimulates the melanosomes to produce
388 melanin. The blood levels of α-MSH are significantly lower in nocturnal-pale geckos (Fig. 8A), so they
389 will consequently produce a little amount of melanin in the dermis (Fig. 8B), which leads to a
390 decrease of the skin's reflectance (Fig. 8C).

391

392



393

394 **Figure 8** - **α-MSH and Melanin assay.** A) α-MSH hormone blood concentration, B) melanin assay in
395 skin, and C) reflectance of the skin in dark-diurnal (D) and pale-nocturnal (N) geckos (one-way
396 ANOVA test $p<0.05$).

397

398 **Discussion**

399 There was a moment in the history of life on Earth when humans began to be decisive for the life of
400 the entire Planet. Three major revolutions shaped human history: Cognitive (70 kya), Agricultural
401 (10 kya), and Scientific (500 ya) revolutions (Harari 2018), which marked a deep watershed between
402 *H. sapiens* before and after this moment. The relationship *H. sapiens* established with animals and
403 plants has reverberated in the ecology and evolution of organisms, up to the present day.

Investigating the events that marked this phase, identifying either the animals and the plants involved, their propensity to change, and the impact of the change, brings new evidence to comprehend the evolutionary processes, the history of life on Earth and ourselves.

The human impact on natural populations can affect their phenotype, playing an important role in evolution. In wild populations, the propensity to establish a relationship with humans was allowed by a plastic capacity, whether domesticated, synanthropic or commensal. Indeed, plasticity confers the ability of niche exploration a prominent potential, especially in order to adapt to the anthropic niche, due to the peculiar speed of its creation and change.

Our results highlight a remarkable variation, both in morphological and functional terms, of the pale-nocturnal gecko's population. The latter derived from the dark-diurnal population, and their common ancestor has been dated to around ca 40,000 years ago. In our hypothesis, starting from that moment, pale-nocturnal geckos would have been differentiated by extending the boundaries of their plasticity. Indeed, their depigmentation went beyond the lightning value that the dark-diurnal gecko could normally be able to achieve. The pale phenotype has been reached through the reduction of α-MSH hormone as well as the number and size of melanosomes in the dermis (reinforcement).

This lightening would have responded to the main selective pressure effective in the dark environment: the highly frequent predation of a nocturnal animal on a light substrate.

However, most likely they would have triggered metabolic implications: a heterothermic lizard can live in the absence of the sun only if it significantly elevates its metabolism, eating more, as pale-nocturnal geckos should have accomplished during evolution. However, this point is worthy of future investigation.

Our study inaugurates a new and interesting line of investigation that could shed light on the impact that humans have on the evolutionary trajectories of non-domesticated species, endowed with adaptive plasticity, functional to the exploration and colonization of the anthropic niche.

The case of the Moorish gecko is emblematic. It clearly describes a species with plastic capacities: its adaptation results from the ability to become cryptic on different substrates. This attitude, in presence of artificially lit environments, would have been translated into the ability to invade the new artificial niche, with the subsequent reinforcement of the plastic ability. This leap would have created the pale-nocturnal population of geckos with a common ancestor when *H. sapiens* deal with managed fire.

The timing of the human control of fire is a controversial issue (Clark and Harris 1985; Gowlett et al. 1981). The European evidence suggests that Neanderthals and early modern humans used the controlled and constant fire starting from the Middle Pleistocene onward in Europe, and from the Late Pleistocene in Europe and South Africa, for many other purposes than just warming and cooking (Henry et al. 2011; Roebroeks and Villa 2011).

The first ideal case of fire documentation, based on a piece of pitch keeping a human fingerprint, is dated at ca 48,000 BP on the limits of techniques and compatible with a geological age of approximately 80,000 years. Although during the Middle Pleistocene, recognizable hearths demonstrate a social and economic focus on many sites attributable to the early hominins.

Interestingly, in that Era there was a clear sympatry between geckos and humans.

Over time, genetic changes have occurred in the nocturnal population, which shifted the coloration from an induced plastic response, by the reduction of melanin aggregation in the dermis, to a lighter coloration, and improved crypticism due to a constitutive reduction of this pigment. Probably, these genetic changes were subsequently increased in frequency in the pale-nocturnal population through positive selection, whereas obviously the pale-nocturnal geckos would be hardly targeted by

predators (Fulgione et al. 2019); consequently, they would be more likely to survive and reproduce, thus passing on the genetic changes underlying the pale phenotype to their offspring.

Plasticity facilitated the initial colonization and survival of the population in a new environment and allowed time for a subsequent genetic adaptation to express a "fine-tune" response to this environment. Plastic capabilities of the phenotype can respond to selection, anticipating the genotype's adaptation process and gaining the colonization of new niches. This new evolutionary perspective, however fascinating, requires accomplishing several empirical tests in species with different plastic capacities.

The gecko brings something new to our attention. Indeed, the challenge the gecko populations have faced is totally unexpected. This is not a new niche, however foreseen by a natural context, the nocturnal niche with artificial light is a niche's new dimension created by humans. A sort of environmental change that follows from those possible foreseen by natural systems.

The light of the fire that pierces the night is not a consequence of the evolution of natural systems, but it's like "pulled-out of a hat rabbit", when nothing suggests this.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions: Conceptualization: D.F. and M.B.; Sample collection: D.F., E.R., V.M., and M.B; Laboratory experiments: V.M., E.R., B.A. and M.B; Analyses of data: D.F., E.R., B.A., A.M., G.G., and M.B; Supervision: D.F.; Writing original draft: D.F., E.R. and M.B.; Review & editing: D.F., V.M., E.R., B.A., A.M., G.G. and M.B. All the authors read and approved the manuscript.

Data Availability

The sequencing data are deposited in GenBank under accession numbers MK275668 - MK275687

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Graphical Abstract

This image was painted by Domenico Fulgione