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From caves to continents: phylogeography and niche shift of an invasive subterranean spider

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1 **From caves to continents: phylogeography and niche shift of an invasive subterranean spider**

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23 **Abstract**

24 Invasive non-native species are recognized as a serious threat to the native biodiversity of the areas they
25 colonize. The subterranean spider *Howaia mogera* (Yaginuma 1972) (syn. *Nesticella mogera*) is considered a
26 highly invasive non-native species rapidly expanding its geographical range from Asia across the European
27 continent and to remote oceanic islands. Due to its preference for moist, dark, and climatically stable habitats,
28 including caves, this spider represents a potential threat to the endemic fauna living in the forest litter and
29 subterranean environments. Nevertheless, the origin, biogeography, patterns of colonization, and niche
30 preference of *H. mogera* remain unexplored. In this study, we reconstructed the phylogeography of the species
31 combining a multi-locus phylogenetic analysis and a niche modeling approach. Our results confirm the center
32 of origin of *H. mogera* in southern China, from where the species naturally spread and diversified in eastern
33 Asia during the Pleistocene Epoch. Its expansion into non-native areas occurred only recently, possibly as a
34 consequence of human-mediated passive transportation and in conjunction with a shift in the habitat preference
35 of the species. Non-native populations have shifted from an original preference for a subterranean lifestyle to
36 more generalist conditions. This change has allowed them to exploit a wider breadth of habitats and has
37 facilitated their expansion in Europe and remote oceanic islands. Yet, the retention of the original subterranean
38 habitat preferences in these non-native populations poses a conservation threat to specialized and fragile
39 subterranean ecosystems, which *H. mogera* can efficiently exploit in invaded areas. Our study underscores the
40 importance of comprehending phylogeography and niche dynamics in invasive non-native species to predict
41 and manage their future expansions. We further highlight the urgency of monitoring *H. mogera*'s spread
42 worldwide, particularly in Southern Europe, to protect fragile invaded ecosystems.

43

44 **Keywords:** biogeography, conservation, fragile ecosystems, invasive non-native species, niche
45 expansion, subterranean habitats.

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49 **INTRODUCTION**

50 Invasive non-native species, namely plants or animals accidentally or intentionally introduced beyond
51 their native range due to human activities and that are spreading rapidly in their invaded range (Soto et al.
52 2023), are recognized as major threats to biodiversity (Mack et al. 2000; Bellard et al. 2016; Early et al. 2016;
53 Pyšek et al. 2020). These opportunistic species are often prone to rapidly adapting to new environments,
54 occupying distinct climatic and ecological niches compared to their original habitats (Broennimann et al. 2007;
55 Zhang et al. 2020; Bujan et al. 2021). Indeed, these species can severely impact ecosystems they invade through
56 competition, predation, pathogen transmission, or hybridization with local species, ultimately altering the
57 composition of native communities (Vilà et al. 2011; Simberloff et al. 2013; Vilà and Hulme 2017; Zhang et
58 al. 2020). Additionally, being often human-transported, non-native species can reach remote habitats located
59 far beyond their normal dispersal range, establishing stable populations that are challenging to manage or
60 eradicate (Vander Zanden et al. 2010; Russell et al. 2017).

61 As drivers of local extinction, the threat posed by invasive non-native species is particularly pervasive
62 in island-like ecosystems, such as oceanic islands and subterranean environments (Wynne et al. 2014; Russell
63 et al. 2017; Nicolosi et al. 2023), which often host communities with limited taxonomic, phylogenetic, and
64 functional redundancy (Gibert and Deharveng 2002). Non-native species can relatively easily invade islands
65 and effectively outcompete native species, leading to disruptions in the structure and functioning of their
66 ecosystems (Simberloff et al. 2013; Russell et al. 2017). Similar to islands, the subterranean realm is a delicate
67 ecosystem highly susceptible to external disturbance (Mammola, Cardoso, et al. 2019). While deep
68 subterranean habitats are generally regarded as significant ecological filters for the establishment of non-native
69 species, an increasing number of biological invasions have been recorded in terrestrial caves, groundwaters,
70 and other subterranean habitats (Nicolosi et al. 2023). These invasions often occur at the surface/subterranean
71 interface, threshold zones often characterized by less selective ecological conditions than deep subterranean
72 habitats. Subterranean species (hereafter termed “troglophiles”, Sket 2008), due to their overspecialization and
73 small populations, can be easily overrun by quickly adaptable and competitive non-native species that may
74 invade their habitats.

75 The rapid increase in biological invasions in remote environments, including caves, has drawn the
76 attention of researchers to the study of the ecology, biogeographic history, and adaptive capacity of non-native
77 species capable of invading biodiversity hotspots (Macisaac et al. 2010; Nicolosi et al. 2023). In this context,
78 modern genetic analysis can serve as a powerful tool for tracking the geographical center of origin of non-
79 native species and delineating their invasive patterns with increasing precision. Generally, pathway analysis is
80 the primary and crucial step in understanding the spread of non-native species (Nentwig 2008; Turbelin et al.
81 2022). Simultaneously, the use of Species Distribution Modeling (SDM) techniques has substantially improved
82 over the last decade, providing powerful tools for tracking non-native species and predicting their potential
83 areas of colonization (Srivastava et al. 2019; Montagnani et al. 2022; Lin et al. 2023; Santamarina et al. 2023).
84 However, the wide majority of modern studies on terrestrial invasive non-native species have been primarily
85 focused on plants, vertebrates, and some charismatic invertebrates, while other taxa have been long neglected
86 (Pyšek et al. 2008). Among the studied invasive organisms, spiders (Arachnida, Araneae) are often
87 underestimated and poorly understood, despite being a significant part of the human-transported and
88 established aliens (Nentwig 2015; Dawson et al. 2017). With the number of invasive spider species rapidly
89 increasing (Nentwig and Kobelt 2010), their evolutionary histories, niche preferences, and the processes that
90 have led to their expansion in sensitive ecosystems remain still poorly understood.

91 The scaffold-web spider *Howaia mogera* (Yaginuma, 1972) (syn. *Nesticella mogera*) (Araneae:
92 Nesticidae), serves as a compelling example of a poorly studied yet widely distributed invasive species with
93 the potential to threaten remote biodiversity hotspots, including both oceanic islands and caves (Howarth 1978,
94 1981; CABI 2023). Considered a species with a preference for moist, dark, and climatically stable habitats
95 (Bielak-Bielecki and Rozwalka 2011), thus with pronounced troglomorphic habits (Yaginuma 1979; Mammola
96 et al. 2018, 2022), *H. mogera* is widely distributed throughout Eastern Asia, ranging from Southern China to
97 Japan. This species occurs both in caves (Liu and Li 2013; Zhang and Li 2013; Ballarin and Li 2018) and in
98 surface and shallow subterranean environments (Yaginuma 1972; Ballarin and Eguchi 2023). Since the 1950s,
99 *H. mogera* has been progressively recorded outside the Asian continent, where it was identified as a potential
100 threat to local biodiversity by replacing native species, particularly in caves (Howarth 1978). Stable
101 populations have been recorded in several oceanic islands including Hawaii, Fiji, St. Helena, Reunion, and
102 possibly São Tomé, as well as in greenhouses and botanical gardens of numerous European cities (Howarth

103 1978; Esyunin et al. 2019; Bloem and Noordijk 2021; World Spider Catalog 2023). Small reproductive
 104 populations have also been found in natural conditions in temperate European Southern and Eastern regions
 105 (Marusik and Guseinov 2003; Pantini et al. 2020).

106 The expanding geographic range, adaptability, and rapid invasiveness, classify *H. mogera* as an invasive
 107 non-native species to the extent that it has been included in the CABI Invasive Species Compendium (CABI
 108 2023). However, to date, the biogeography, dispersal patterns, and evolutionary history of this spider remain
 109 largely unexplored. In this study, we elucidate the historical biogeography of *H. mogera*, with the following
 110 objectives: i) comprehensively reconstructing a time-calibrated phylogeography of the species, ii) uncovering
 111 its center of origin and time and mode of its invasive dispersal pattern in Europe and oceanic islands, and iii)
 112 investigating the species ecological preferences while estimating its potential future areas of expansion beyond
 113 its natural range, including in critical biodiversity hotspots.

114

115 **MATERIAL and METHODS**

116 **Taxon sampling, phylogenetic analysis, and haplotype network reconstruction**

117 A total of 88 *H. mogera* specimens were collected from 60 sites across Eastern Asia, Europe, and remote
 118 Pacific and Indian Ocean islands. Our sampling efforts aimed to cover the species' entire known geographic
 119 range, including native and non-native populations. We harvested additional gene sequences from GenBank
 120 (<https://www.ncbi.nlm.nih.gov/genbank/>), Dryad (<https://datadryad.org/stash>), or unpublished data derived
 121 from previous studies (Ballarin and Li 2018). Additional *Howaia* species were included as outgroups. The tree
 122 was rooted using *Nesticella kaohsiungensis* Lin, Ballarin and Li, 2016 based on previous phylogenetic
 123 reconstructions (Ballarin and Li 2018; Ballarin and Eguchi 2023). Table S1 in Supporting information lists all
 124 the used specimens.

125 We amplified three gene partial fragments, two mitochondrial [*cytochrome c oxidase 1 (COI)* and *16S*
 126 *ribosomal RNA (16S)*] and one nuclear [*28S ribosomal RNA (28S)*] genes. Primer pairs and protocols details
 127 are outlined in Supporting information and in Table S2. We performed Bayesian Inference (BI) on MrBayes
 128 v.3.2.7 (Ronquist et al. 2012) using two independent runs of four Carlo Markov chains (MCMCs) for 20

129 million generations following the partition scheme by Partitionfinder 2, sampling every 2000 generations with
 130 a burning fraction of 25%. We assessed chain stabilization and convergence using Tracer v.1.7.1 (Rambaut et
 131 al. 2018) ensuring an effective sample size (ESS) >200 for all the parameters. We ran Maximum Likelihood
 132 (ML) analysis on RAxML v.8.2.12 (Stamatakis 2014), as twice 1,000 rapid bootstrap replicates under the
 133 GTRGAMMA model with a random starting tree. We performed both analyses through the online CIPRES
 134 Science Gateway v. 3.3 (Miller et al. 2010). We inferred the unique haplotypes frequency of each gene and
 135 concatenated sequences with DnaSP v.6.12 (Rozas et al. 2017). The resulting haplotype networks were
 136 visualized using PopArt (Leigh and Bryant 2015).

137

138 **Divergence time and ancestral area reconstruction**

139 *Divergence time*

140 We inferred divergence time with BEAST v.1.10.4 (Suchard et al. 2018) under an uncorrelated relaxed
 141 lognormal clock. For input, we used a unique haplotypes dataset derived from concatenated genes to prevent
 142 coalescent and zero-length branches. We defined four subsets and substitution models as reported by
 143 PartitionFinder 2. The tree models were set unlinked. To test the potential impact of different models, we
 144 conducted two separate analyses using either Yule or Birth-Death tree priors. Due to the lack of fossils of
 145 *Howaia* or closely related genera, we used standard gene substitution rates derived from previous studies on
 146 spiders (Bidegaray-Batista and Arnedo 2011; Bidegaray-Batista et al. 2014). Although not specifically
 147 designed for Nesticidae, these rates have been extensively utilized for time-calibrate spider phylogenies,
 148 including the superfamily Araneoidea to which *Howaia* belongs (McHugh et al. 2014; Čandek et al. 2019).
 149 We excluded Paleogeographic events used for time calibrations in previous studies on Asian nesticids (e.g.,
 150 formation of the Yangtze River, Zhang and Li 2013) to prevent circular reasoning. The mitochondrial genes
 151 (*COI*, *16S*) were linked under the same clock, the distribution was set as normal with $uclid.mean = 0.0132$ and
 152 standard deviation = 0.0015 (Bidegaray-Batista et al. 2014). We performed a comparative test setting a normal
 153 distribution with $uclid.mean = 0.0112$ and standard deviation = 0.001 (Bidegaray-Batista and Arnedo 2011). In
 154 both cases, the nuclear gene (*28S*) was set as uniform with an initial value of 0.00115 and min/max bounds of
 155 0.0001 and 0.0115, respectively (Xu et al. 2015). For each analysis, we performed twice four independent

156 MCMC runs of 50 million generations sampling trees every 5,000 generations, discharging 10% of resulting
 157 trees as burn-in. Convergence was checked with Tracer v.1.7.1. The final trees were combined using
 158 TreeAnnotator v.1.10.4 and edited in FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/>).

159

160 *Ancestral range reconstruction*

161 We utilized the BioGeoBEARS package (Matzke 2013) implemented in R (R Core Team 2021) to infer
 162 the ancestral range of *H. mogera* under the dispersal-extinction-cladogenesis (DEC) model. The unique
 163 haplotype time-calibrated tree from the BEAST analysis was used as input. We avoided the founder-event
 164 speciation parameter (+J) model due to recent critiques suggesting possible overestimations (Ree and
 165 Sanmartín 2018). Further tests using the AIC and AICc criteria suggested DEC as the most fitting model for
 166 the analysis. Based on the current distribution of *H. mogera* we defined five regions: Japan (A), North China
 167 (B), Central-South China (C), Taiwan (D), and non-native (E), the latter representing areas of potential
 168 introductions. The maximum number of regions was set as two since the known range of each *H. mogera*
 169 population or other species used in the analysis does not exceed this value.

170

171 **Species distribution modeling**

172 *Occurrence database*

173 We assembled a dataset of 201 occurrence localities for *H. mogera* based on literature and field surveys
 174 published up to 2021 (Supporting information Table S3). Our genetic analysis delineated individuals from the
 175 two main clades as geographically distinct, consequently, we assigned each native record to either Clade 1
 176 (Central-South China; n = 49) or Clade 2 (North China and Japan; n = 84). The remaining 68 occurrence points
 177 belonged to non-native populations (“alien”) spread across East Asia, Europe, and oceanic islands. To mitigate
 178 spatial sampling heterogeneity among the localities and prevent an overestimation of presence occurrences
 179 (Mammola 2017) we aggregated data within cells corresponding to the resolution of the environmental
 180 predictors in all spatial analyses.

181

182 *Accessible area*

183 We calibrated the model within the accessible area, specifically the geographical extent that we
184 hypothesized the species to have occupied throughout its evolutionary history (i.e., native range) (Barve et al.
185 2011). The natural dispersal ability of *H. mogera* is currently unstudied, consequently, we assumed a dispersal
186 potential comparable to other cave-dwelling spiders adapted to cave entrances (Mammola 2017; Mammola
187 and Isaia 2017), creating a buffer area for each clade extending 500 km around the presence records.
188 Additionally, we delimited the calibration area southward for Clade 1 and northward for Clade 2 by the
189 Yangtze River since it represents the most significant natural obstacle to the dispersal of *H. mogera* and other
190 similar species in their native ranges (Ballarin and Li 2018) (Supporting information Fig. S6).

191

192 *Environmental predictors*

193 We selected the initial set of predictors through an expert-based selection and refined this set using
194 statistical inference (see Dormann et al. 2013; Fourcade et al. 2018; Mammola and Leroy 2018). To represent
195 the surface and subterranean conditions across the species distribution, we employed topographical (altitude),
196 geological (availability of karst rock), and bioclimatic variables. We obtained all variables at a resolution of
197 2.5 arc-minutes. We sourced a standard elevation data raster layer from the WorldClim dataset (Hijmans et al.
198 2005). We extracted climatic variables (average annual temperature, minimum temperature of the coldest
199 month, temperature annual range, cumulative annual precipitation, average annual precipitation, and solar
200 radiation) similarly from WorldClim2 (Fick and Hijmans 2017). These variables are proxies for the conditions
201 of habitats at the surface/subterranean interface (Mammola 2017; Mammola, Aharon, et al. 2019). We obtained
202 a shapefile of carbonate outcrops for the study area from the World Map of Carbonate Rock Outcrops (v.3.0).
203 We rasterized the shapefile and calculated the area of each karst patch following Mammola, Aharon, et al.
204 (2019), assigning the area value of the corresponding karst patch-value to each raster pixel, and a value of 0
205 for non-karst pixels.

206 Lastly, we checked multicollinearity among predictors (Braunisch et al. 2013) *via* pairwise Pearson's
207 correlation coefficients (r), and kept only one among highly correlated predictors (threshold for collinearity at
208 Pearson $|r| > 0.70$, Dormann et al. 2013). We based the choice of which variable to retain on our expert opinion,
209 namely keeping those variables more ecologically meaningful in representing species biology and life history.

210

211 *Species distribution prediction*

212 We developed SDMs for Clade 1 and Clade 2 using the maximum entropy modeling approach (Maxent),
213 a machine learning algorithm commonly used to predict species habitat suitability when lacking reliable
214 absence data (Phillips et al. 2006). Given that Maxent requires background data (Phillips et al. 2006;
215 VanDerWal et al. 2009), for each clade, we randomly selected 10,000 points within the calibration area
216 (Supporting information Fig. S1). Previous studies have indicated that the default settings in Maxent may
217 impair model predictive abilities (Warren and Seifert 2011; Muscarella et al. 2014). Hence, we automatically
218 optimized the Maxent regularization multiplier and feature class for each clade using the *ENMevaluate*
219 function in the ENMeval R package, adopting a 5-fold random cross-validation (Kass et al. 2021). As with
220 Zhang, Kass et al. (2021), for each clade, we selected the optimal Maxent model based on both the 10th
221 percentile omission rate and area under the receiver operating characteristic curve (AUC) (Swets 1988).
222 Among the 25% quantile of omission rate, we selected the Maxent model with the highest validation AUC
223 value as the optimal model. We further estimated model predictive performance of the two optimal Maxent
224 Models via two evaluation metrics [namely true skill statistics (TSS) (Allouche et al. 2006) and the continuous
225 Boyce index (Boyce et al. 2002; Hirzel et al. 2006). We determine the permutation importance of each predictor
226 and estimated their response curves. We converted continuous predictions of habitat suitability into binary
227 maps using 10th percentile presence probability threshold (Zhang, Kass et al. 2021).

228

229 **Niche overlap**

230 We used geometrical n -dimensional hypervolumes (Blonder et al. 2014, 2018) to model the ecological
231 niche of Clade 1, Clade 2, and non-native populations. We delineated each hypervolume using the non-

232 collinear variables introduced in the SDMs, with a Gaussian kernel density estimator and bandwidth values
 233 estimated with the cross-validation method (Blonder et al. 2018). We standardized variables prior to
 234 hypervolume construction. We assessed hypervolume similarity using both an overlap and a distance metric
 235 (Mammola 2019), namely the distance between hypervolume centroids and their pairwise overall
 236 differentiation, as estimated with the *kernel.beta* R function (Mammola and Cardoso 2020). We finally used
 237 the *kernel.dispersion* R function (Mammola and Cardoso 2020) to calculate hypervolume dispersion for native
 238 and non-native populations, thereby assessing potential niche expansion in the invaded range.

239

240

241 RESULTS

242 Phylogeny and population diversity

243 The final concatenated gene sequences yielded 3,352 nucleotides: *COI* = 1,200 bp, *16S* = 482 bp, *28S*
 244 = 1,670 bp while the complete dataset included 94 terminals. No tree typology differences were found between
 245 the BI and ML (analyses Fig. 1c and Supporting information Figs. S1, S2) and BEAST analysis of unique
 246 haplotypes (Fig. 2b). All major clades were highly supported. The resulting trees strongly support *H. mogera*'s
 247 monophyly and affinity with congeners (BI = 1, ML = 99), as in past studies (Ballarin and Li 2018; Ballarin
 248 and Eguchi 2023). All methods highlight the presence of two main distinct clades within *H. mogera* (BI = 1,
 249 ML = 99) with a disjunct distribution. One large lineage (Clade 1) includes all populations from Central-South
 250 China approximately distributed south of the course of the Yangtze River (Hainan, Hubei, Guangxi, Guizhou,
 251 Sichuan, Yunnan provinces, and Taiwan). All sub-clades, including specimens from non-native areas, are
 252 located in a distal position within Clade 1 showing a close relationship with endemic South Chinese
 253 populations and, in some cases, being mixed with them. Individuals from Denmark, Germany, Italy, Russia,
 254 the Balearic Islands, and Fiji Islands all belong to the same monophyletic lineage, being a sister clade to
 255 populations from Guangxi. Specimens from Reunion Islands are embedded within the lineage of the Chinese
 256 populations from Guangxi and Yunnan. The other main clade (Clade 2) consists of two distinct sub-clades (BI
 257 = 1, ML = 87) formed respectively by populations distributed in Japan and Centre-North China, roughly north

258 of the Yangtze River. Such results are in line with a recent analysis of the Japanese populations of *H. mogera*
259 (Ballarin and Eguchi 2023).

260 The number of unique haplotypes identified by the DnaSP analysis changed with the considered gene:
261 *COI* = 30, *16S* = 17, *28S* = 6. (Fig. 1b and Supporting information Figs. S3–S5). In the concatenated dataset,
262 31 unique haplotypes were detected (Fig.1b). Haplotype networks support the separation among Japanese,
263 Taiwanese, North, and South Chinese populations obtained from the phylogenetic analysis. Haplotypes of non-
264 native populations are closely related or mixed with haplotypes from South Chinese populations. Populations
265 from Europe, China, and Oceanic islands share the same haplotype: Hap10 (Guangxi, Yunnan, and Reunion
266 Islands), Hap12 (Balearic Islands, Denmark, Germany, Italy, Fiji Islands), and Hap14 (Balearic Islands, Fiji
267 Islands, and Russia).

268

269 **Divergence time and historical biogeography**

270 The estimated divergence time and ancestral range reconstruction of *H. mogera* phylogeny are
271 illustrated in Fig. 2b. No significant differences in time estimation for the ingroup nodes were found among
272 different tests. We estimate that the divergence of the species from other congeners occurred at approximately
273 6.5 Ma, with a 95% highest posterior density interval (HPD) of 9.2–4.6 Ma. Results of the BioGeoBEARS
274 analysis suggest Southern China as the center of origin of the species, with a possible expansion to the northern
275 areas during the Pliocene Epoch (5.33–2.58 Ma). A major vicariance event occurred at approximately 2.27 Ma
276 (HPD = 3.3–1.5 Ma) and is reported as the probable cause of the separation between Clade 1 from Clade 2.
277 The colonization of the Japanese archipelago occurred from Northern China by populations belonging to Clade
278 2 during the Pleistocene (2.58 Ma–11.7 Ka). Starting from approximately 0.4 Ma (HPD = 0.68–0.2 Ma), the
279 Japanese and North Chinese lineages split from each other and continued to evolve separately up to the present
280 day. Over the past 1 Ma, a major diversification occurred within the populations of Clade 1 leading to the
281 gradual emergence of several sub-lineages in Central-South China. Our analysis suggests a South Chinese
282 origin for all these sub-lineages, including non-native populations. These sub-clades are among the youngest
283 lineages in the tree, with their origin estimated within the last 0.15–0.9Mya.

284

285 **Species habitat suitability prediction**

286 Based on collinearity analyses, we selected annual precipitation, minimum temperature of the coldest
 287 month, karst area, and solar radiation as predictors for modeling the species distribution (Figs. 3a–d). The
 288 evaluation metrics demonstrated that the optimal Maxent model for each clade exhibited good predictive
 289 abilities with AUC over 0.8, TSS over 0.6, and continuous Boyce index over 0.5 (Supporting information
 290 Table S4). Our results suggest that the four predictors have different importance in regulating the distribution
 291 patterns of the two native clades: solar radiation is probably the most important for Clade 1, while annual
 292 precipitation is critical for Clade 2 (Supporting information Fig. S7). Response curves further show that the
 293 two clades have different environmental requirements (Fig. 3a). Model predictions suggest that the suitable
 294 range for Clade 1 in Asia is primarily concentrated in the southwest part of China (Fig. 3b). A considerable
 295 portion of Japan and Korea is predicted to be suitable for Clade 2. All these results align with recent studies
 296 on the distribution of *H. mogera* (Ballarin and Eguchi 2023). Notably, the analysis failed to identify known
 297 presence records of Clade 2 in China, but suggested the regions near the Yangtze River as probably suitable
 298 for this clade (Fig. 3b). Additionally, our analysis forecasted potential suitable habitats for Clade 1 in various
 299 parts of the world, particularly in Central Africa, South America, South Eastern Australia, New Zealand, the
 300 Caspian Sea region, and Southern Europe (Fig. 3c). In Europe, the projected potential distribution frequently
 301 coincided with areas abundant in karst formations (Fig. 3d).

302

303 **Niche overlap**

304 The 4-dimensional hypervolume representing the niche of Clade 2 exhibited greater volume compared
 305 to Clade 1 (6.34×10^{12} versus 1.43×10^{15}) (Fig. 4e). The two niches displayed minimal overlap in the
 306 multidimensional space ($\beta_{\text{total}} = 0.99$; Distance between centroids = 169771). We estimated that this niche
 307 differentiation was primarily due to processes of niche contraction/expansion ($\beta_{\text{difference}} = 0.99$) rather than
 308 a replacement of niche space between the two clades ($\beta_{\text{replacements}} = 0.0005$). In terms of realized niche, non-
 309 native populations were more closely associated with Clade 1 ($\beta_{\text{total}} = 0.86$; Distance between centroids =

10805), aligning with the genetic profile of the populations, rather than Clade 2 ($\beta_{\text{total}}= 0.99$; Distance between centroids=158995). In general, the hypervolume of non-native populations exhibited greater dispersion (26422 versus 2359 of Clade 1 and 226634 of Clade 2), suggesting niche expansion within the invaded range.

314

DISCUSSION

Phylogeography of *H. mogera* in East Asia

Here, we present a detailed case study of a highly invasive, yet poorly studied troglomorphic spider capable of colonizing both surface and subterranean ecosystems. Although *H. mogera* has long been considered a species of Asian origin, such an assumption has never been tested empirically (Ballarin and Eguchi 2023). Our study reconstructs the phylogeny and biogeography of this spider, confirming its monophyly and East Asia as the native range of the species (Fig. 1c). We further identify the Central-South Chinese region as its most likely center of origin (Fig. 2b), and estimate that the separation of *H. mogera* from its congeners occurred approximately 6.5 Ma. Such a result is in line with the time of origin of *H. mogera* estimated in more comprehensive studies on short-legged nesticids [~ 6.0 Ma, HPD = ~ 8.0 – 4.0 Ma in Zhang and Li (2013); ~ 7.0 Ma, HPD = ~ 9.5 – 5.0 Ma in (Ballarin and Li 2018)]. We set the main split event, and consequent formation of the two major lineages of *H. mogera* at approximately 2.27 Ma, at the end of the Pliocene-Pleistocene transition (3.3–2.4 Ma). The main reason for this cladogenetic event has been linked to the final formation of the Yangtze River in Central China but has never been proven by empirical methods (Zhang and Li 2013; Ballarin and Li 2018). Our results corroborate this explanation. Although the timeframe of the gorge formation is strongly debated and some researchers backdate the origin of the Yangtze River as far back as 23 Mya (Zheng 2015), several studies suggest a younger age (Zhang et al. 2021). Allegedly, the river and its related gorge underwent significant modifications and reshaping during the late Pliocene and possibly early Pleistocene, which led to the establishment of the current modern course (Liu et al. 2018; Liu et al. 2019). It is possible that this late geological rearrangement of the river acted as a major geographic barrier for *H. mogera* in Central China, dividing the populations north and south of the gorge and limiting their gene flow. Allopatry may also have been promoted by the climate cooling and consequent local vegetational shift that occurred in East Asia during

337 the same period (Huang et al. 2001; Yang and Ding 2010; Herzsuh et al. 2016; Li et al. 2019). Similar to
338 other animal species (Fu and Wen 2023), Pleistocene climatic events may have caused a general resettlement
339 of *H. mogera* populations in East Asia, promoting intraspecific diversification and leading to the local
340 segregation of the numerous lineages in Central-South China, North China, and Japan (Fig. 2b).

341

342 **Dispersion outside the native range**

343 *Howaia mogera* is considered a highly invasive species with a pronounced capacity for dispersion and
344 adaptation to novel environments (Esyunin et al. 2019). While atypical among nesticid spiders, such adaptive
345 traits can be occasionally observed in species belonging to this family (Vink and Duperre 2011; Nardi et al.
346 2023). Our analysis supports the hypothesis of recent human-driven colonization of *H. mogera* beyond its East
347 Asian native range. This geographical expansion involved only haplotypes originally distributed in Southern
348 China (Clade 1), as all the non-native populations belong to this lineage (Fig. 1b, c). Such event likely occurred
349 through several independent colonization events involving distinct South Chinese populations from the
350 Yunnan and Guizhou Provinces (Fig. 2a, b). Although Clade 1 demonstrates marked troglophilic preferences
351 (Fig. 4c, see also Ballarin and Eguchi 2023), non-native populations belonging to this lineage have been
352 observed inhabiting a very diverse array of surface environments within invaded areas. Our findings indicate
353 that this heightened level of habitat tolerance is a direct consequence of a remarkable niche expansion
354 involving the introduced populations (Fig. 4e). The occurrence frequency and importance of niche expansion
355 in invasive species is still debated, possibly being less common than expected (Liu et al. 2020). However, in
356 the case of *H. mogera*, the remarkable adaptability of the South China populations enabled their successful
357 expansion into various environments, both natural and artificial. In contrast, lineages from North China and
358 Japan (Clade 2) despite their stronger surface affinity (Fig.3c, see also Ballarin and Eguchi 2023) did not
359 undergo a similar niche adaptation (Fig. 4a–e). Consequently, their distribution remained confined to their
360 native range despite having a larger predicted suitable habitat worldwide compared to Clade 1 (Supporting
361 information Fig. S8).

362 It is widely recognized that human activities play a significantly role in the dispersal of invasive species
363 (Nentwig 2008; Early et al. 2016). For arthropods, including spiders, global trade, and specifically the transport

364 of soil and live plants, is frequently recognized as primary factor in the spread, introduction, and establishment
365 of alien species (Kobelt and Nentwig 2008; Rabitsch 2010; Pergl et al. 2017; Meurisse et al. 2019). Containers
366 or potted plants, by offering stable humidity and temperature, create favorable microhabitats that can
367 temporarily shelter spiders, allowing even species with particular microhabitat preferences to be effectively
368 transported to distant locations (Nentwig 2015). We suggest that this may also be the case for *H. mogera*. In
369 Europe, adults of this species have been frequently found in greenhouses or other indoor habitats (Bloem and
370 Noordijk 2021), often associated with ornamental plants, particularly orchids imported from China to the
371 Netherlands (Bielak-Bielecki and Rozwalka 2011; Lissner pers. comm.).

372 The trade in Chinese orchid plants has likely actively spread *H. mogera* across Europe, since these
373 plants are commonly used in greenhouses or as indoor decorations. Additionally, the transportation of plants,
374 soil, or other goods by ship from South China may have served as the primary pathway for the establishment
375 of *H. mogera* in remote oceanic islands (Fig. 2a). How *H. mogera* reached remote islands with no apparent
376 trade connections with Eastern Asia (e.g., Reunion Islands) remains unclear. Such cases can be attributed to
377 secondary trade routes established between these islands and European countries where *H. mogera* may be
378 already present.

379

380 **Future of colonization and expected impact on the local fauna**

381 The increasing number of documented records in non-native regions in the last 50 years (Supporting
382 information Table S3) strongly suggests that *H. mogera* is a species undergoing rapid geographic expansion.
383 Our study indicates that this trend is likely to persist in the future. Based on our findings, we have identified a
384 broad range of suitable non-native regions worldwide where, if reached, the species can potentially establish
385 permanent colonies. These regions include continents, areas, and islands where its presence has yet to be
386 documented, such as eastern Black Sea, Central Africa, South America, New Zealand, the southeastern coasts
387 of Australia, and the northwestern coast of North America. (Fig. 3c). Given the rapid adaptation and niche
388 expansion observed in *H. mogera*, coupled with the ongoing growth of global trade, it is highly likely that this
389 species will colonize at least some of these areas in the near future.

390 In Europe *H. mogera* is essentially a eusynantropic species (Hänggi et al. 2021) relying on artificial
391 warm indoor conditions to survive during the cold European winters (Esyunin et al. 2019). Nevertheless, recent
392 records of this species in natural conditions have been reported in central and Southern Italy (Pantini et al.
393 2020; this work) and in caves in Southern Spain (Ribera, personal comm.). Such new observations, which
394 include juveniles and females with egg cocoons (Fig. 1d), provide evidence of potential external establishments
395 where more favorable climatic conditions persist throughout the year. Stable populations of *H. mogera* in
396 natural or semi-natural conditions can thus be expected to become more frequent in Central-south Europe in
397 the future, a phenomenon that can be further exacerbated by climate change. A similar trend can also be
398 observed among other invasive spider species in Europe with similar ecological traits to *H. mogera*
399 (Narimanov et al. 2022; Nardi et al. 2023). In this expansive colonization scenario, a particular concern arises
400 regarding the South-European karst areas which frequently overlap with the geographic areas estimated to be
401 suitable for *H. mogera* (Fig. 3d). As a troglomorphic species, *H. mogera* still retains the capacity to exploit
402 subterranean environments. Previous records of stable colonization in non-native subterranean ecosystems
403 have been observed in Hawaiian caves (Gertsch 1973; Howarth 1978) where predation and competition
404 pressure have ultimately led to the replacement of some native arthropod species (Howarth 1978, 1981; CABI
405 2023). The new findings in Spanish caves suggest that similar events may occur also in Europe. As a generalist
406 predator capable of establishing abundant populations, *H. mogera* may pose a significant threat to the local
407 subterranean fauna, as previously observed in the Hawaiian caves. Based on such conclusions, a proper effort
408 to monitor the presence and expansion of *H. mogera* in fragile ecosystems should be considered a priority in
409 areas where the presence of the species is already documented or may be expected. Particular emphasis should
410 be placed on monitoring oceanic islands or other hotspots of diversity and endemism, where the negative effect
411 of invasive species is estimated to be the highest.

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Conclusions

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Our study shed new light on the ecological dynamics of troglomorphic invasive species with adaptability to both surface and subterranean habitats. We confirm *H. mogera* as native to Eastern Asia, where it underwent

417 a natural diversification during the Pleistocene epoch. The recent dispersion of the species worldwide can be
418 attributed to a combination of different factors, including niche expansion, rapid adaptation, and human-
419 mediated transportation likely related to plant trades. This combination of factors has rapidly propelled *H.*
420 *mogera* far beyond its natural range and original habitat, enabling the successful colonization of a wide range
421 of different natural and artificial environments. Consequently, our findings help understanding niche dynamics
422 in invasive non-native species with troglophilic preferences. The ability of *H. mogera* to invade and establish
423 permanent populations in delicate ecosystems, such as remote oceanic islands or caves, poses a consistent
424 threat to the local fauna, making its monitoring and containment a conservation priority. In particular, we
425 emphasize the need for proper monitoring of its expansion in Southern Europe, where future invasions in the
426 local subterranean habitats are expected.

427

428

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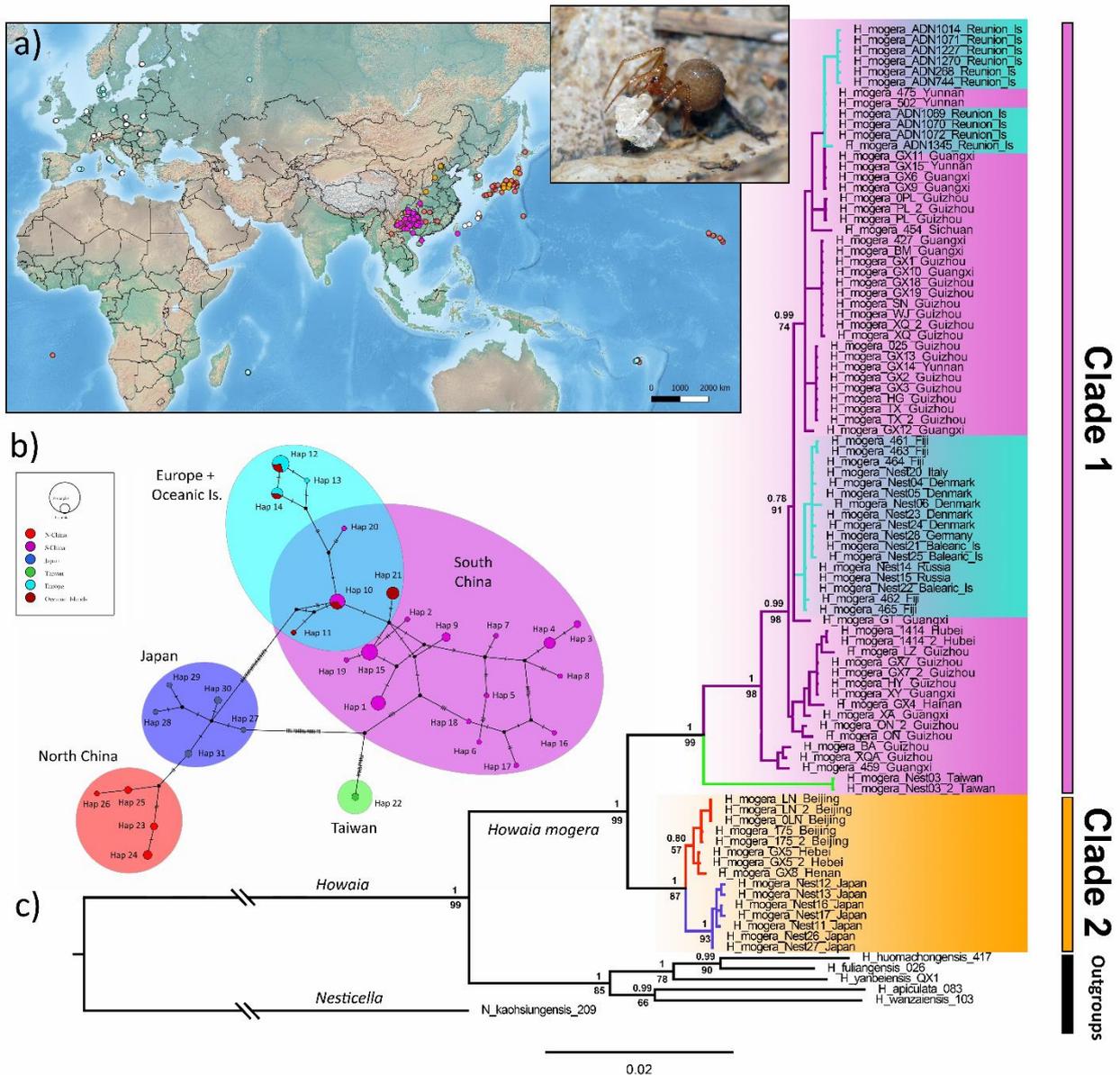
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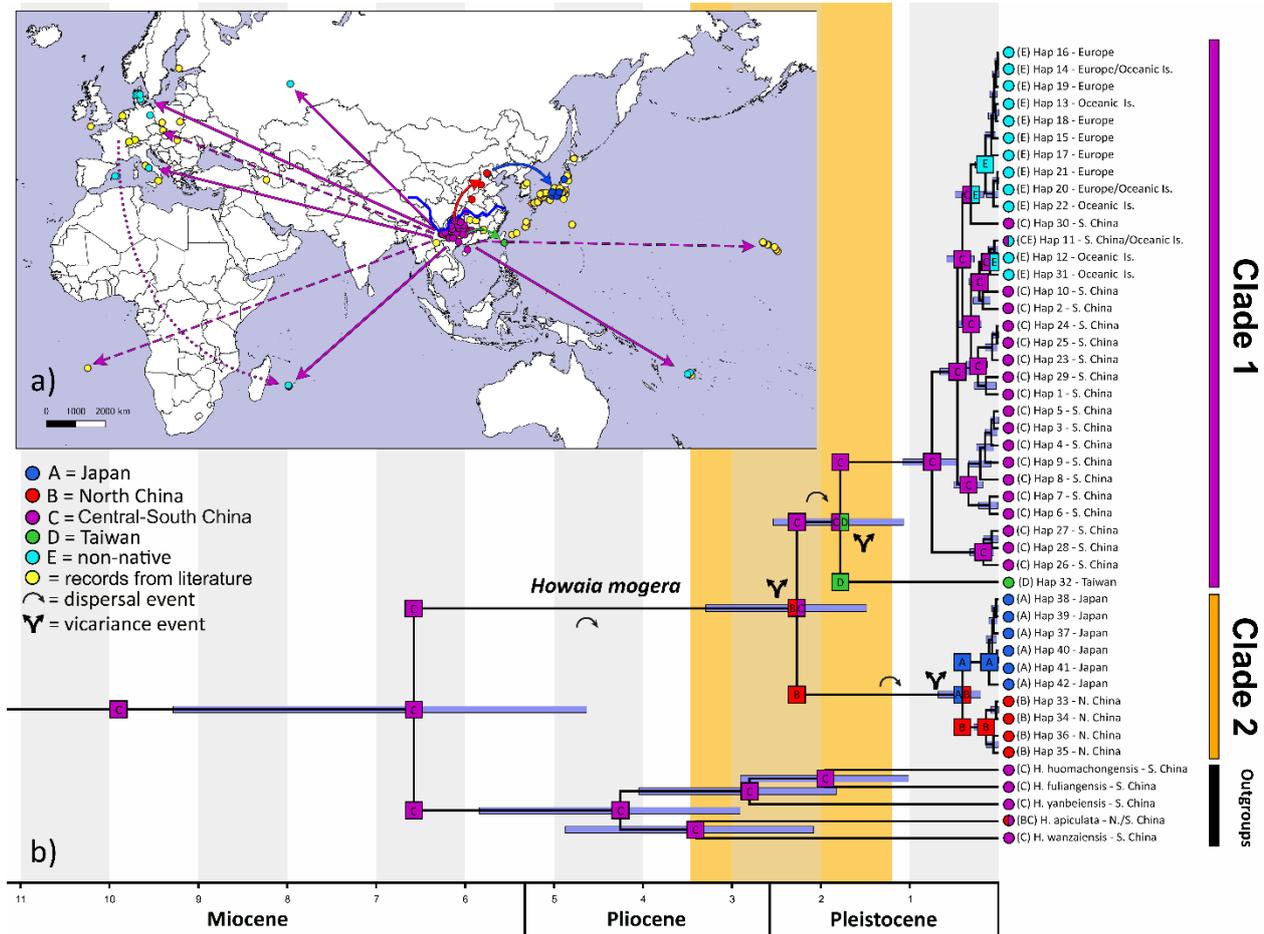
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Figure 1. Distribution and phylogeny of *Howaia mogera*. (a) Map of distribution of *H. mogera*: white dots show known localities from bibliography, other colours refer to specimens used in this study. The two main clades are indicated in magenta (Clade 1) and orange (Clade 2), the azure colour refers to non-native specimens. (b) Haplotype network of *H. mogera* based on unique haplotypes dataset of all genes concatenated. Colors refer to different geographical populations as reported in the related legend, the size of circles is proportional to the haplotype frequency. (c) Phylogenetic tree of *H. mogera* based on all concatenated genes, main clades are highlighted as in the map. The colors of the branches indicate subclades from different geographical areas: blue = Japan, red = North China, green = Taiwan, magenta = South China, and azure = non-native (Europe, oceanic islands). Branch lengths are scaled to the number of substitutions per site. The

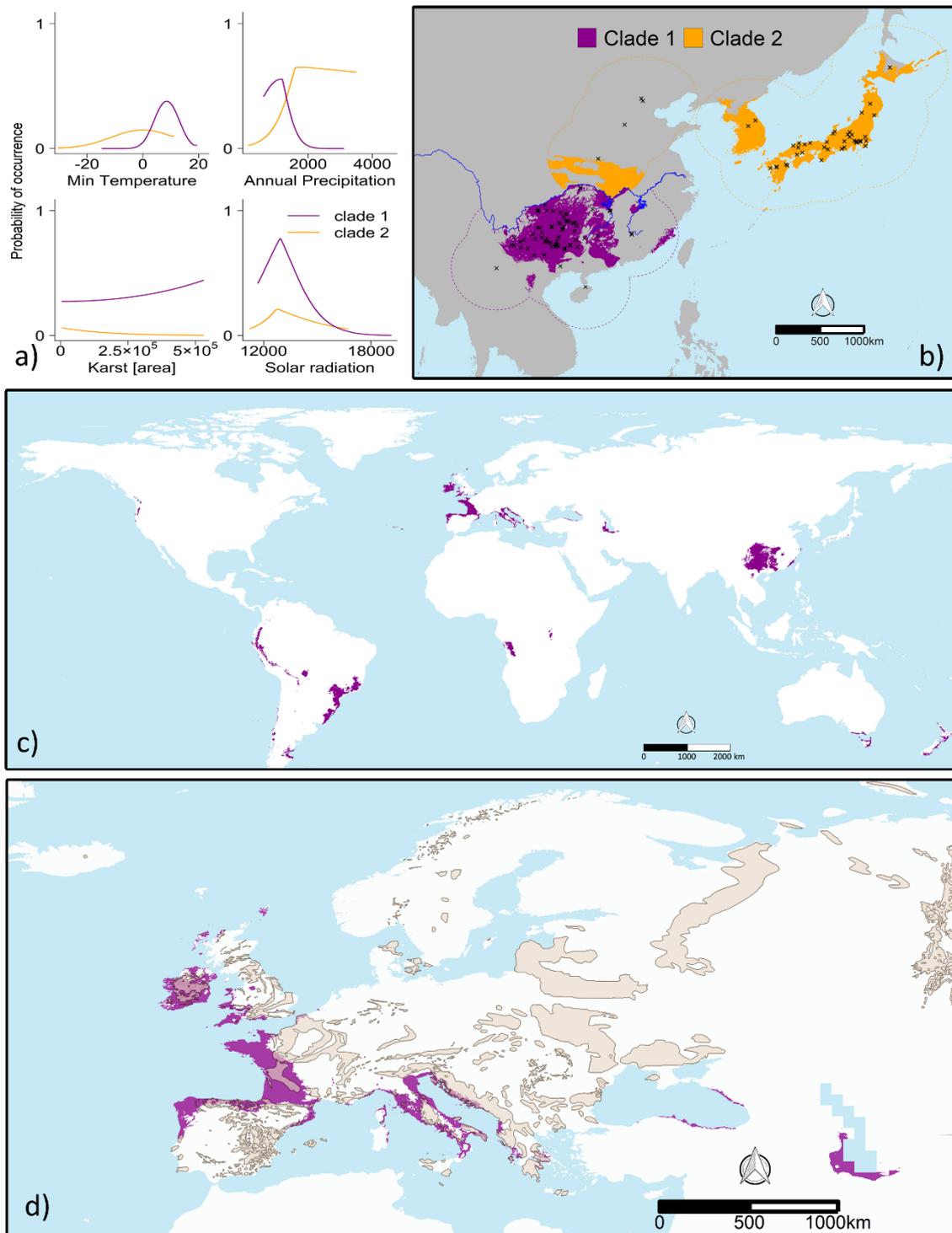
756 branch of the root species *Nesticella kaoshungensis* is truncated as indicated by slashes. Numbers at nodes
 757 represent the related Bayesian posterior probability (upper) and Maximum Likelihood bootstrap (lower)
 758 support values. The photo shows an adult female of *H. mogera* with an egg cocoon photographed in natural
 759 conditions in central Italy. Photo by Luigi Lenzini.



760

761 Figure 2 Historical biogeography of *Howaia mogera*. (a) Map of distribution of *H. mogera* and proposed
 762 dispersal routes as discussed in the text. Yellow dots refer to literature records, other colours refer to different
 763 populations as reported in the map. Coloured arrows show routes of introduction of non-native populations
 764 (magenta) or historical dispersal routes (other colors) of the original clades, dashed arrows indicate probable
 765 invasive routes not directly confirmed by the molecular analysis, dotted arrow shows a possible alternative
 766 introduction route to Reunion Islands. (b) Time-calibrated ancestral area reconstruction inferred with
 767 BioGeoBEARS software on the base of the unique haplotypes output derived from BEAST. The single most
 768 probable ancestral range is mapped at each node. Corner positions represent geographic ranges immediately
 769 after a cladogenetic event. Blue bars at nodes indicate 95% credibility interval of divergence time estimates.

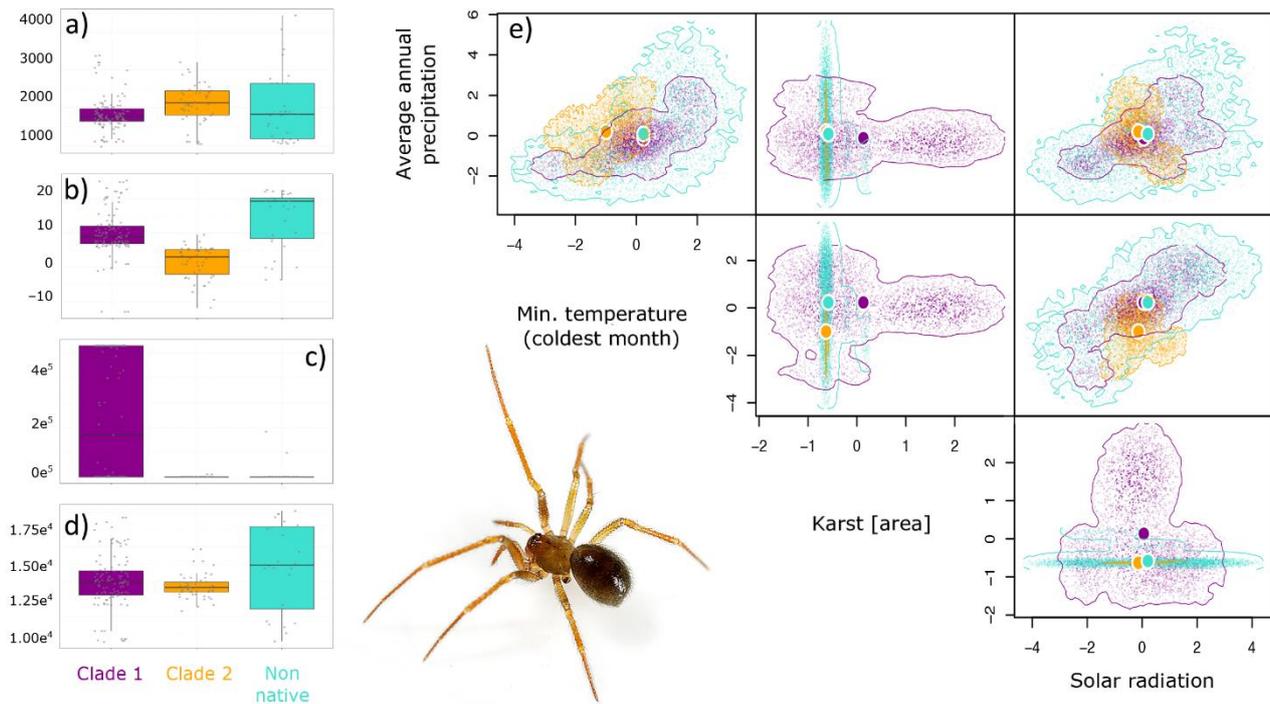
770 Dispersal and vicariance events are represented by a curved or a forked arrow respectively. The orange area
 771 shows the younger estimated timeframe of the final assessment of the Yangtze River according to the literature.



772

773 Figure 3. Maxent analysis results for two clades (dark magenta for Clade 1, orange for Clade 2) of *H.*
 774 *mogera*. (a) Response curves of two clades to environmental factors discussed in the text. Numbers mean
 775 permutation importance. (b) Binary habitat suitability predictions in native ranges, blue lines represent the

776 Yangtze River and black crosses indicate species presence records, dashed lines mean calibration area. (c)
 777 estimated worldwide suitable area of distribution of *H. mogera*'s Clade 1 (dark magenta) as resulting from the
 778 Maxent analysis. (d) Detail of the estimated suitable area of distribution of *H. mogera* Clade 1 (dark magenta)
 779 in Europe as reported by the Maxent analysis. The distribution is overlapped to the Karst regions present in
 780 Europe (light brown areas) (modified after COST 65 1995).



781

782 Figure 4. Environmental conditions experienced by the populations of *H. mogera* (Clade 1, Clade 2,
 783 and non-native populations) (a–d) and a representation of the niche of the three clades (e). (a) Mean annual
 784 precipitations. (b) Mean temperature of the coldest month. (c) Karst area. (d) Solar radiation. The grey dots
 785 represent extracted values. (e) Pairplots showing the 4-dimensional hypervolumes for the Clade 1, Clade 2,
 786 and non-native populations of *H. mogera*. The colored points reflect the stochastic points sampled from the
 787 inferred hypervolumes rather than original observations (5,000 random points for each hypervolume are
 788 shown).