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► To cite this version:

S Renaud, Emilie Hardouin, Pascale C Chevret, Katerina Papayiannis, Petros Lymberakis, et al.. Morphometrics and genetics highlight the complex history of Eastern Mediterranean spiny mice. *Biological Journal of the Linnean Society*, 2020, 130 (3), pp.599-614. 10.1093/biolinnean/blaa063 . hal-02988720

HAL Id: hal-02988720

<https://hal.science/hal-02988720v1>

Submitted on 21 Nov 2020

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1 **Morphometrics and genetics highlight the complex history of Eastern Mediterranean spiny mice**

2

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24 Short running title:

25 *Acomys cahirinus* in the Eastern Mediterranean area

26

27 **Abstract**

28 Spiny mice of the *Acomys cahirinus* group display a complex geographic structure in the Eastern
29 Mediterranean area, as shown by former genetic and chromosomal studies. In order to better
30 elucidate the evolutionary relationships of insular populations from Crete and Cyprus with the
31 continental ones from North Africa and Cilicia in Turkey, genetic and morphometric variations were
32 investigated, based on mitochondrial D-loop sequences, and size and shape of the first upper molar.
33 The Cypriot and the Cilician populations show idiosyncratic divergence in molar size and shape, while
34 Crete presents a geographic structure with at least three differentiated sub-populations, as shown by
35 congruent distributions of haplogroups, Robertsonian fusions and morphometric variation. A
36 complex history of multiple introductions is most probably responsible for this structure, and insular
37 isolation coupled with habitat shift should have further promoted a pronounced and rapid
38 morphological evolution in molar size and shape on Crete and Cyprus.

39

40 **Keywords**

41 *Acomys minous*; *Acomys cillicicus*; *Acomys nesiotus*; Crete; Cyprus; D-loop; geometric morphometrics;
42 insular evolution; molar shape; phylogeography.

43

44 Introduction

45 Spiny mice of the genus *Acomys* occupy a large distribution area over Africa to Western Asia,
46 including a small area along the Mediterranean coast of Turkey, a region historically known as Cilicia.
47 They further occur on the islands of Crete and Cyprus, within the Eastern Mediterranean area, which
48 along with Cilicia, constitute the northernmost distribution limit of the genus. The lack of clear
49 diagnostic characters led to a taxonomic debate within a group of closely related species known as
50 the *cahirinus-dimidiatus* group (Denys et al., 1994; Volobouev et al., 2007; Frynta et al., 2010; Aghová
51 et al., 2019). A growing body of genetic and chromosomal evidence showed that one clade,
52 attributed to *A. dimidiatus*, nowadays occupies the Levant, including Israel and Sinai, up to Arabia
53 and Iran. The other clade (*A. cahirinus*) would only occur between Eastern Sahara and Egypt along
54 the Nile Valley (Volobouev et al., 2007; Frynta et al., 2010). Spiny mice from Crete, Cyprus and Cilicia
55 are affiliated to the second group (Barome et al., 2001; Frynta et al., 2010; Giagia-Athanasopoulou et
56 al., 2011). Being considered endemics, and some of them displaying distinct morphological features,
57 such as a larger body size (Kryštufek & Vohralík, 2009), each of these populations was given a specific
58 or subspecific status: *A. nesiotetes* for the Cypriot, *A. cilicicus* for the Cilician, and *A. minous* for the
59 Cretan populations. Crete, however, is characterized by genetic heterogeneity, with the co-existence
60 of two distinct mitochondrial clades, one also found in Cyprus and the low Nile valley (Cairo, Egypt),
61 and the other one in Cilicia (Barome et al., 2001) and the high Nile Valley, Libya, and Chad (Frynta et
62 al., 2010). Cretan mice further vary in their chromosomal number, which ranges from $2n=38$ to
63 $2n=42$, due to Robertsonian fusions. No individual association has been found between the
64 chromosomal number and the mitochondrial lineage (Giagia-Athanasopoulou et al., 2011).

65 In order to better understand the evolutionary history of the *cahirinus* “*sensu lato*” group (including
66 *A. minous*, *A. nesiotetes* and *A. cilicicus*), a geometric morphometric analysis of the first upper molar
67 (Fig. 1) was performed. In *Acomys* as in murine rodents, the first molar erupts before weaning and is
68 subsequently affected only by wear. Molar shape differences are thus indicative of underlying
69 genetic changes. In contrast, osteological characters, such as skull or mandible, are prone to plastic
70 remodelling along an animal’s life and consequently, they also vary on a short time-scale, depending
71 on local ecological conditions (Caumul & Polly, 2005; Ledevin et al., 2012). Furthermore, molar teeth
72 are the most frequent fossil remain of small mammals; their study allows a comparison of modern
73 and ancient samples, providing a temporal perspective. Hence, the morphometric analysis of molar
74 shape may deliver valuable insight on the genetic differentiation among populations, complementary
75 to the analysis of mitochondrial DNA for which the existing data were supplemented with new
76 mitochondrial D-loop sequences from Crete, Cyprus and Cilicia.

77 Based on these morphometric and genetic data, the following questions were addressed: (1) Is the
78 genetic and chromosomal heterogeneity on Crete mirrored in a high morphological disparity? (2) Can
79 a synthesis of the genetic, morphometric and chromosomal data shed light on the processes that led
80 to the diversity within *Acomys cahirinus s.l.* in the Eastern Mediterranean area? (3) Insularity is
81 known to trigger rapid morphological divergence (Millien, 2006). If drift in isolated populations is the
82 dominant factor, a pronounced morphological divergence is expected in Cilicia, as on the islands of
83 Crete and Cyprus. If adaptation to island-specific ecological conditions, including changes in the level
84 of competition and predation, is the prime driving factor of divergence (Lomolino, 1985, 2005), the
85 Cilician population should display less divergence compared to those of Crete and Cyprus.

86

87 **Material**

88 *Sampling for genetics*

89 Thirty new D-loop sequences were acquired, including specimens from Cyprus, Crete, and Cilicia
90 (Table 1): (1) Five Cypriot specimens ("*A. nesiotus*") collected in 2015. Despite a sampling effort
91 around the island, specimens were only caught on Cap Greco or adjacent to it. (2) Eleven Cretan
92 specimens ("*A. minous*"). (3) Fourteen Cilician individuals ("*A. cilicicus*") trapped in spring 2013,
93 around Narlıkuyu, in the district of Mersin (Turkey).

94

95 *Sampling for morphometrics*

96 The morphometric study (Table 1) included 92 specimens collected in Crete (61), Cyprus (6), and
97 Cilicia (17). This sampling was complemented by eight specimens from Cairo (Egypt) from the
98 Museum National d'Histoire Naturelle de Paris (vouchers: 2001-11; 1997-1308; 1996-432; 1996-446;
99 1996-431; 1996-430; 1994-1280; 1999-6), identified as *A. cahirinus*.

100 Four other specimens from the Museum National d'Histoire Naturelle de Paris were attributed with
101 less certainty to *A. cahirinus*, but coming from Sudan and Chad, they allowed to document the
102 geographic variation at a larger scale (vouchers: 1906-118a; 1906-118b; 1906-118c; 1981-1059).

103 Body size and sex data were available for most specimens, allowing to investigate the occurrence of
104 sexual dimorphism and allometry. All specimens were identified as sub-adult or adult, based on the
105 criterion of the eruption of the third molars. One specimen from Cairo (Egypt) was a juvenile, since
106 its third molars were not erupted. It was not included in the analyses of body size, but since the

107 molar does not grow further after its eruption, it was included in the analyses of first molar size and
108 shape.

109 Finally, three fossil teeth from the floor of the Hellenistic Temple C at the ancient port of Kommos on
110 the south coast of Crete, dated between 375 B.C. and AD 160/170 (Shaw, 2000), were measured
111 based on drawings of published plates (Payne, 1995) and included in the morphometric analyses.

112

113 **Methods**

114 *Genetic analyses*

115 *Acomys* tissue samples were extracted using DNEasy from Qiagen following the manufacturer
116 instructions. A D-loop fragment of 514 bp was amplified using previously described primers (Nicolas
117 et al., 2009). PCR conditions were as follows: 10ng of DNA, 2mM of MgCl₂, 0.2mM dNTP, 0.5U of
118 Taq, 0.2 μM of each primer. PCR cycles were 15 min 95°C, followed by 35 cycles with 30 sec at 95°C,
119 1:30 min at 54°C, 1 min at 72°C; final elongation was 15 min at 72°C. The sequences generated were
120 visualized and analyzed CLC Workbench (Qiagen) and aligned with Seaview v4 (Gouy et al., 2010).
121 Genetic diversity indices were calculated using DNAsp (Librado & Rozas, 2009).

122 A phylogenetic tree was generated using MrBayes (Ronquist et al., 2012) and PhyML 3.0 (Guindon et
123 al., 2010), including the 30 sequences generated in the present study and the 16 sequences available
124 from GenBank, leading to a total of 46 sequences of *A. cahirinus s.l.*. The haplotypes were
125 determined with DNAsp (Librado & Rozas, 2009). Only the 20 haplotypes differing by mutation were
126 retained for the phylogenetic analysis. We added to these haplotypes seven *A. dimidiatus* (AJ012028,
127 MH044889, MH044888, MH044871, MH044868, MH044840, FJ415545) as well as three *A. ignitus*
128 (MH044872, MH044875, MH044876), two *A. wilsoni* (MH044862, MH044874) and three *A. russatus*
129 (MH044881, MH044885 and FJ415546) that were used as outgroups. The sites with more than 20%
130 missing data were removed and the final alignment comprised 35 sequences and 493 sites. The
131 substitution model, GTR+I+G, was chosen using jmodeltest (Darriba et al., 2012). Robustness of the
132 nodes was estimated with 1000 bootstrap replicates with PhyML and posterior probability with
133 MrBayes. The generation number was set at 2000000 MCMC with one tree sampled every 500
134 generations. The burn-in was graphically determined with Tracer v1.7 (Rambaut et al., 2018). We also
135 checked that the effective sample sizes (ESSs) were above 200 and that the average standard
136 deviation of split frequencies remained <0.01 after the burn-in threshold. We discarded 20% of the
137 trees and visualized the resulting tree with Figtree v1.4 (Rambaut, 2012). A median-joining haplotype
138 network was constructed in PopART (Leigh & Bryant, 2015) with the 46 *A. cahirinus* sequences only.

139 All the sequences generated in the present study are available on GenBank (GenBank numbers
140 MT001830-MT001858, MT043301) (Table 1).

141 To estimate the divergence time of the lineages of *A. cahirinus* in the Eastern Mediterranean area,
142 we used BEAST v 2.5.2 (Bouckaert et al., 2019), including its functions BEAUTI, LogCombiner and
143 TreeAnnotator. The mitochondrial (Cyt *b* + D-loop) and nuclear genes (IRBP, RAG1) were retrieved
144 from GenBank for 32 *Acomys* representing the main lineages within this genus (Aghová et al., 2019)
145 and 4 outgroups (*Deomys ferrugineus*, *Lophuromys flavopunctatus*, *Lophuromys sikapusi* and
146 *Uranomys ruddi*) (Supp. Table 1). The two Eastern Mediterranean lineages were each represented by
147 one specimen for which sequences of the four genes were available (Supp. Table 1). The dataset
148 comprised 36 sequences and 3509 bp. The best partitioning scheme and substitution models were
149 determined with PartitionFinder 2 (Lanfear et al., 2017) using a greedy heuristic algorithm with
150 'linked branch lengths' option and the Bayesian Information Criterion (BIC) (Supp. Table 2). The
151 partitions were imported in BEAUTI, where they were assigned separate and unlinked substitution
152 and clock models. Bayesian analyses were run with uncorrelated lognormal relaxed clocks, birth-
153 death tree prior and three fossil constraints defined by using lognormal statistical distributions. We
154 used the fossil constraints proposed by Aghová et al. (2019) within the genus *Acomys*, and hence the
155 same specifications of lognormal priors: offset of 8.5 Ma for the most recent common ancestor
156 (MRCA) of the genus *Acomys*, 6.08 Ma for the MRCA of the clade encompassing *cahirinus* + *wilsoni* +
157 *russatus* and 3 Ma for the MRCA of the *spinosissimus* group (Aghová et al., 2019); mean = 1 in the
158 three cases (Aghová et al., 2019). Two independent runs were carried out for 50 million generations
159 with sampling every 1000 generations in BEAST. The first 10% were discarded as burn-in and the
160 resulting parameter and tree files were examined for convergence and effective sample sizes in
161 Tracer 1.7 (Rambaut et al., 2018). The two runs were combined in LogCombiner and the species tree
162 was visualized in TreeAnnotator.

163

164 *Morphometrics analyses*

165 First upper molars (UM1) were photographed using a Leica MZ 9.5 binocular, being manually
166 oriented so that the occlusal surface matched at best the horizontal plane. The shape of the UM1
167 was described using 64 points sampled at equal curvilinear distance along the 2D outline of the
168 occlusal surface using the Optimas software. An outline-based method was chosen, because reliable
169 landmarks are difficult to position on murine-like molars. The top of the cusps is abraded by wear
170 and cannot be used for assessing the position of the cusps, and landmarks bracketing the cusps on

171 the outline are difficult to position, given the smooth undulations delineating the cusps along the
172 outline. The starting point was tentatively positioned at the anterior-most part of the tooth.

173 The points along the outline were analysed as sliding semi-landmarks (Cucchi et al., 2013). Using this
174 approach, the outline points are adjusted using a generalized Procrustes superimposition (GPA)
175 standardizing size, position and orientation, while retaining the geometric relationships between
176 specimens (Rohlf & Slice, 1990). During the superimposition, semi-landmarks were allowed sliding
177 along their tangent vectors until their positions minimized the shape difference between specimens,
178 the criterion being bending energy (Bookstein, 1997). Because the first point was only defined on the
179 basis of a maximum of curvature at the anterior-most part of the UM1, some slight offset might
180 occur between specimens. The first point was therefore considered as a semi-landmark allowed to
181 slide between the last and second points.

182 The centroid size (CS) of the 64 points (i.e. square root of the sum of the squared distance from each
183 point to the centroid of the configuration) was considered as an estimate of overall tooth size.

184 Differences between groups were tested using an analysis of variance (ANOVA) and relationships
185 between variables were assessed using Pearson's product-moment correlation. The pattern of tooth
186 shape differentiation was explored using multivariate analyses of the aligned coordinates. A principal
187 component analysis (PCA on the variance-covariance matrix of the aligned coordinates) allowed a
188 first exploration of the dataset. It was complemented by a between-group PCA (bgPCA). While the
189 PCA is an eigenanalysis of the total variance-covariance of the dataset, the bgPCA analyses the
190 variance-covariance between group means weighted by the sample size of each group.

191 Size-related variations in shape and differences between groups were investigated using Procrustes
192 ANOVA. With this approach, the Procrustes distances among specimens are used to quantify the
193 components of shape variation, which are statistically evaluated via permutation (here, 9999
194 permutations) (Adams & Otarola-Castillo, 2013). The allometric relationship was visualized as the
195 common allometric component (CAC) (Adams et al., 2013). To assess the impact of allometry on the
196 pattern of shape differentiation, a bgPCA was also performed on the residuals of a regression of the
197 aligned coordinates vs. centroid size.

198 The GPA and the Procrustes ANOVA were performed using the R package *geomorph* (Adams &
199 Otarola-Castillo, 2013). The PCA and the bgPCA were performed using the package *ade4* (Dray &
200 Dufour, 2007). For ANOVA and Procrustes ANOVA, the grouping factor was considered to be the
201 region (see Table 1), in order to increase sample size and ameliorate the performance of the tests.
202 For the bgPCA, the grouping factor was the trapping locality. It corresponds to field data, and the

203 clustering of neighbouring localities in the morphospace is indicative of geographic structure, despite
204 low sample size for small groups.

205

206 **Results**

207 *Genetic analyses*

208 As the phylogenetic trees reconstructed with MrBayes and PhyML were congruent, only the Bayesian
209 tree is presented (Fig. 2). Two main lineages were found with good support (BP > 0.75, PP > 0.45)
210 within *A. cahirinus s.l.*. Lineage A [according the terminology of (Barome et al., 2001)] consisted of
211 haplotypes from Western and Central Crete, Cyprus, Cairo (Northern Egypt) and Chad. Lineage B
212 involved samples from Eastern and Central Crete, Cilicia, Southern Egypt and Libya. The sequences of
213 Chad and Libya appear to be basal with regards to the two lineages, and they were attributed neither
214 to lineage A nor to B.

215 Both D-loop and cytochrome *b* showed two lineages within *A. cahirinus s.l.* and the two
216 mitochondrial genes provided congruent outcomes at the individual level [(Barome et al., 2001;
217 Frynta et al., 2010); this study]. One exception regarded the specimen R155 from Piskopiano (Crete)
218 published with a *cyt b* sequence belonging to lineage B (Giagia-Athanasopoulou et al., 2011) and for
219 which we found a D-loop belonging to lineage A. We re-sequenced both genes and confirmed the D-
220 loop attribution to lineage A. Another discrepancy regarded the North African specimens, which
221 were attributed to lineage B for the *cyt b* (Frynta et al., 2010). Based on the present D-loop results,
222 they rather appear to have a basal position in the phylogenetic tree with regards to lineages A and B.

223 Eastern and Central Crete shared haplotypes with Cilicia. Western and Central Crete shared other
224 haplotypes with Cyprus; none were in common between Cyprus and Cilicia (Fig. 2). This result is
225 confirmed by the *F_{st}* being lower between Cyprus and Crete (*F_{st}* = 0.28, *p* = 0.01) and between Cilicia
226 and Crete (*F_{st}* = 0.60, *P* < 0.001) than between Cyprus and Cilicia (*F_{st}* = 0.83, *P* < 0.001). Haplotype
227 diversity was found to be higher in Crete (*H_d* = 0.864) than in Cyprus (*H_d* = 0.667) or Cilicia (*H_d* =
228 0.448).

229 In the network (Fig. 3), a finer geographic structure could be recognized, especially in Crete. The few
230 African samples appeared as central in the network, with the exception of Cairo, which appeared to
231 be divergent but nested within lineage A. Haplotypes from Crete appeared intermediate between the
232 basal African haplotypes and either those of Cyprus (for lineage A) or those of Cilicia (for lineage B). A
233 geographic structure emerged in Crete, with different haplotypes found in the West, Centre and East
234 of the island. Within lineage A, a central haplotype (in blue on Fig. 3) was found in both Western

235 Crete and in Cyprus; other haplotypes were either characteristics of Western or Central Crete.
236 Eastern Crete was characterized by a haplotype affiliated to lineage B, also present in the
237 easternmost locality of Central Crete (Stalida Mochos) and in Cilicia. Cilicia further hosted two
238 specific haplotypes, while Cyprus displayed three specific haplotypes.

239 Based on a combined analysis of mitochondrial (Cyt *b* + D-loop) and nuclear genes (IRBP, RAG1), the
240 divergence between the two lineages was estimated at 210 000 years [110 000 - 320 000] (Supp.
241 [Figure 1](#)).

242

243 *Morphometric analysis of the first upper molar differentiation*

244 Sexual dimorphism

245 Data on sex and body size (head + body length) were available for most specimens. Body size, as well
246 as UM1 centroid size, were well differentiated among regions, but not between sexes ([Table 2](#)).
247 Tooth shape was also very different among regions, while sexes were only weakly differentiated. As a
248 consequence, males and females were pooled in subsequent analyses, to focus on geographical
249 patterns of differentiation.

250 Geographic variations in size and relationship between body and tooth size

251 Body size varied greatly among but also within populations ([Fig. 4A](#)). This variation is partly due to
252 the age structure of the populations, since very young animals can be trapped, as shown by the
253 specimen without erupted third molars, which displays a very small body size. Specimens from
254 Western Crete tended to display the largest body size, whereas those from Cilicia were among the
255 smallest. Animals from Cyprus and Cairo tended to have a large body size.

256 This trend was not reflected in the pattern of molar size variation ([Fig. 4B](#)). Animals from Western
257 Crete, while being among the largest, displayed small molar teeth. Similarly, the large animals from
258 Cairo displayed extremely small teeth, while the spiny mice from Cyprus, of similar body size,
259 displayed among the largest teeth of the dataset. As a consequence, body and molar size were not
260 related ([Fig. 4C](#)). In a model including region and body size as explanatory variables, UM1 centroid
261 size varied greatly among regions ($P < 2e-16$) but only weakly with body size ($P = 0.0158$).

262 Overall, tooth size displayed a reduced variation within localities and within regions, in contrast to
263 body size, but the differences in molar size were important even among regions of Crete. Spiny mice
264 from Saharan regions (Sudan and Chad) displayed important variations in molar size, even within

265 Sudan, despite the fact that these mice were all derived from Khartoum. The fossil teeth from
266 Kommos were of large size, similar to the modern Central and Eastern Cretan populations.

267

268 Tooth shape differences among populations

269 The different regions were highly different in shape (Procrustes ANOVA: $P < 0.0001$). In the
270 morphospace defined by the first two axes of the PCA on the aligned coordinates (Fig. 5A),
271 populations from Western and Eastern Crete were opposed along PC1. This axis describes a change
272 from teeth with receding anterior cusps leading to an elongated anterior part, to teeth with
273 prominent, anteriorly shifted lingual cusp t1 and labial cusp t3 (Fig. 5B). Negative scores along PC2
274 characterize teeth from Cyprus, with a broader and rounder posterior part, closer labial cusps (t3 and
275 t9), an anteriorly shifted lingual cusp t1, and an anterior part compressed on the lingual side (Fig. 5C).

276 A bgPCA (Fig. 5D) provided further insight into the geographic clustering on Crete. The populations of
277 Western Crete appeared as the most differentiated. The Central and Eastern Cretan populations
278 were relatively close to each other. The Cilician populations appeared central in this morphospace.
279 Along the second axis of the bgPCA, Cyprus appeared as highly divergent. At the opposite side of this
280 axis, the two African samples shared high positive scores; the sample from Cairo was closer to
281 Western Crete along bgPC1, whereas the Saharan sample was closer to Central and Eastern Crete.
282 The populations from Central Crete varied along bgPC2, the two samples from Piskopiano and
283 surroundings displaying positive bgPC2 scores, whereas the three other localities shared negative
284 bgPC2 scores. The fossil teeth from Kommos plotted close to the samples from Sahara and
285 Piskopiano in Central Crete.

286

287 Allometric tooth shape variation

288 Since tooth size variation appeared to be important (Fig. 4), allometry was investigated as a source of
289 shape differentiation. Shape appeared as significantly related to size (Procrustes ANOVA: $p = 1e-04$).
290 The Common Allometric Component (CAC) based on this analysis (Fig. 6A) showed a general size-
291 shape trend which may contribute to the shape similarity between the populations with small molars
292 (Cairo, Western Crete) and to those sharing large molar size (Eastern Crete and Cyprus). Large teeth
293 tended to display a prominent and anteriorly shifted first lingual cusp (t1) and a prominent posterior
294 labial cusp (t9) compared to small teeth (Fig. 6B).

295 The size-free shape variation was explored based on a bgPCA on the residuals of a regression of the
296 aligned coordinates on centroid size (Fig. 6C). The pattern of between-population differentiation was
297 not deeply modified compared to the analysis on the raw aligned coordinates (Fig. 5D).

298

299 Discussion

300 *Two ancient haplogroups in Crete*

301 This study confirms the complex geographic structure observed in earlier studies within the Eastern
302 Mediterranean spiny mice *Acomys cahirinus s.l.* (Barome et al., 2001; Frynta et al., 2010; Giagia-
303 Athanasopoulou et al., 2011). Different haplogroups occur in Cyprus and Cilicia, the only area where
304 *Acomys cahirinus s.l.* occurs on a Northern Mediterranean coast, while Crete hosts both haplogroups
305 (Barome et al., 2001; Frynta et al., 2010). The divergence of the two haplogroups has been dated
306 here at 210 kyrs, an estimate based on four genes, several calibration points within *Acomys*, and a
307 Bayesian approach, that allows to re-evaluate the previous estimate of 400 kyrs (Barome et al.,
308 2001). Such a range of dating of divergence is typical for phylogeographic lineages isolated during the
309 Plio-Pleistocene climatic fluctuations [e.g. (Nicolas et al., 2008; Ben Faleh et al., 2012; McDonough et
310 al., 2015)]. Since a Pleistocene fossil record of *Acomys* is absent from Cyprus and Crete (Barome et
311 al., 2001), the divergence between the haplogroups clearly predates the dispersion in this area.

312

313 *A complex geographic structure in Cretan spiny mice*

314 The two mitochondrial lineages have different geographic distributions: lineage A dominates in
315 Western and Central Crete, whereas lineage B occurs mostly in Eastern Crete (Barome et al., 2001;
316 Frynta et al., 2010; Giagia-Athanasopoulou et al., 2011). The present study further shows a finer
317 geographic structure, based on the repartition of the D-loop haplotypes: Western and Central Crete
318 tend to display different haplotypes within lineage A (Fig. 3). This is in complete agreement with the
319 morphometric results, showing different molar size (Fig. 4) and shape (Fig. 5) in Western, Central and
320 Eastern Crete; possibly, a finer differentiation occurs even within Central Crete, with the area around
321 Piskopiano displaying slightly different molar shape (Fig. 5). A reconsideration of the former
322 chromosomal results (Giagia-Athanasopoulou et al., 2011) also suggests regional variations in Crete
323 (Fig. 7), with high, ancestral, diploid chromosome numbers (up to $2n = 42$) dominating in Central
324 Crete, whereas lower, derived diploid chromosome numbers (e.g. $2n = 38$) are found in Western and
325 Eastern Crete (Fig. 7). All markers, despite being very different in their nature (mitochondrial DNA,

326 karyotypes, molar size and shape) are therefore congruent and point to a strong geographic
327 structure in Crete.

328 Nevertheless, evidence of mixing occurs. Regarding the mitochondrial lineages, specimens attributed
329 to lineage B can occasionally be found in regions dominated by lineage A: one mouse in Akrotiri
330 Peninsula, Chania (Barome et al., 2001) and one mouse in the easternmost locality of Central Crete,
331 Stalida Mochos [(Giagia-Athanasopoulou et al., 2011); this study]. Regarding karyotypes, a specimen
332 with the derived diploid chromosome number $2n = 38$ was found in Central Crete, despite the
333 dominance there of high diploid chromosome numbers ($2n = 42$, $2n = 40$ and their hybrids). The
334 occurrence in Eastern Crete of both, homozygous mice with $2n = 40$ and heterozygotes with $2n = 39$
335 suggests the existence in the area of homozygous mice with $2n = 38$, although they have not yet
336 been captured [Fig. 7, (Giagia-Athanasopoulou et al., 2011)].

337 Despite this evidence of mixing, the strong geographic structure observed for molar shape shows
338 that gene flow is not enough to homogenize the populations across Crete. Crete is characterized by a
339 mountainous relief compartmented by basins, with several massifs culminating above 2000 meters.
340 Both high mountains and basins may constitute barriers to dispersal for spiny mice preferably
341 inhabiting Mediterranean environments with rocky substrates (Kryštufek & Vohralík, 2009). This
342 complex geomorphology probably promoted isolation and divergence of *Acomys* populations in
343 different parts of the island.

344

345 *Cretan spiny mice within the diversity of the Eastern Mediterranean area*

346 The diversity observed in Cretan spiny mice is difficult to insert into a general pattern, because the
347 diversity on the African continent is largely under-sampled. However, the distribution of the two
348 haplogroups in the Eastern Mediterranean area points to a complex history of migration. The
349 haplotypes found in Crete, Cyprus and Cilicia were shown to be derived, compared to the haplotypes
350 sampled so far in Libya, Chad, and the high Nile Valley. Chadian and Libyan specimens even appeared
351 to be outside from lineage A or B, due to their basal position, or to incomplete lineage sorting. In
352 contrast, lineage A haplotypes sampled in Cairo appeared to be derived and branched with
353 sequences from Cyprus and Western Crete [(Frynta et al., 2010); this study].

354 Regarding the karyotype evolution, *Acomys* is characterized by an important variation of the diploid
355 chromosome number, ranging from $2n = 68$ to $2n = 40-42$ and even $2n = 38-36$ in the *A. cahirinus*
356 group, knowing that $2n = 36$ is the karyotype with the lowest chromosome number that can be
357 achieved in spiny mice through Rb fusions (Lavrenchenko et al., 2011). Within *A. cahirinus*, $2n=40-42$

358 karyotypes can therefore be considered as 'ancestral' and those of $2n = 36-38$ as 'derived'. In
359 particular, Central Crete hosts ancestral karyotypes ($2n = 40$ and even $2n = 42$). Cyprus displays an
360 additional Robertsonian (Rb) fusion ($2n = 38$), that is also present in Western Crete and possibly in
361 Eastern Crete. A further Rb fusion has led to $2n = 36$ in the Cilicia population and in the Egyptian
362 population from Cairo (Macholán et al., 1995; Zima et al., 1999; Giagia-Athanasopoulou et al., 2011).
363 However, it is difficult to assess the variation within *A. cahirinus* in the African continent, due to a
364 limited geographic sampling and taxonomic uncertainty.

365 Regarding molar morphometrics, the population from Central Crete appears close to the Saharan
366 group and to the fossil teeth from Kommos. Altogether, this suggests that Central Crete may host a
367 population characterized by ancestral tooth morphology, karyotypes, and haplotypes. The Cretan
368 fossils were deposited during the lifetime of the Hellenistic temple of Kommos, which lasted from
369 375 BC to AD 160/170. Since no *Acomys* fossils were ever found in older Cretan deposits (Katerina
370 Papayiannis pers. obs. January 2020), this places the earliest appearance of *Acomys* on Crete during
371 this time interval.

372 The occurrence of a second mitochondrial lineage in Eastern Crete (lineage B), however, suggests
373 that multiple introductions occurred from different continental source populations. Important trade
374 across the Eastern Mediterranean area continued throughout the Bronze Age and Historic times
375 (Karetsou et al., 2001). Likely, the dispersion of *Acomys* through the Eastern Mediterranean area
376 occurred by human-mediated transport (Barome et al., 2001) as unintentional stowaway on boat
377 cargos, in a manner similar to that described for the Western house mouse *Mus musculus domesticus*
378 (Cucchi, 2008). Both Cilicia and Cyprus spiny mice display more derived haplotypes, karyotypes, and
379 tooth shape than those from Crete; this further suggests that Crete acted as a hub from which spiny
380 mice were translocated.

381 In support of this statement, the fact that the $2n = 38$ karyotype of some Cretan spiny mice is
382 identical to the one of Cypriot mice, and differs only by an additional Rb fusion from the $2n = 36$
383 karyotype of Cilician mice, could be explained through the expansion of mice with $2n = 38$ from Crete
384 to the other two regions, irrespective of mitochondrial lineages. The two lineages are reported to
385 freely hybridize in the laboratory (Frynta et al., 2010) and in Crete, they are not associated with
386 specific karyotypes, e.g. there exist spiny mice with $2n = 38$ attributed to lineage A or B (Giagia-
387 Athanasopoulou et al., 2011). Thus, populations with $2n = 38$ could have been imported to Cilicia
388 from Eastern Crete (dominated by lineage B) and to Cyprus from Central or Western Crete
389 (dominated by lineage A). The additional Rb fusion, mentioned above, would have then occurred
390 locally in the isolated population of Cilicia, leading to its very derived karyotype ($2n = 36$).

391 The population from Cairo constitutes a puzzling case. It displays the same derived karyotype as
392 Cilicia ($2n = 36$ with the same Rb fusions), however belongs to lineage A, with haplotypes related to
393 those of Cyprus and Western Crete. Thus, a direct relationship between the Cairo and Cilician spiny
394 mice is unlikely. Moreover, the tooth morphology of the Cairo mice is close in size and shape to the
395 one observed in Western Crete. These facts could be reconciled if the population from Cairo is
396 actually derived from a secondary import on the African continent, from Western Crete or Cyprus.

397 In this case, the identical karyotype of $2n = 36$ in both Cilicia and Cairo would be the result of the
398 independent, *in situ* fixation of the same Rb fusion in the karyotype of both populations. Derived
399 from $2n = 38$, there are only three acrocentric chromosomes available for a new Rb fusion, one of
400 which is very small (Giagia-Athanasopoulou et al., 2011). Rb fusions do not happen completely
401 randomly between acrocentric chromosomes, but similarly-sized chromosomes seem to be
402 preferably fused (Gazave et al., 2003), and this may have triggered the independent fixation of the
403 same Rb fusion in the two, otherwise, distant populations (Lavrenchenko et al., 2011). As in the
404 house mouse, successive Rb fusions, leading to very low diploid numbers, might have been favoured
405 by successive dispersion events, and small patchy distribution (Auffray, 1993). The peculiarity of the
406 Cairo population could be maintained by behavioural mechanisms, similar to those reported for
407 house mouse populations from Tunisia (Chatti et al., 1999), since this population is characterized by
408 its commensal habit (Kryštufek & Vohralík, 2009) that may maintain its isolation from the
409 surrounding populations. Any further interpretation is hampered by the limited data available from
410 the African continent.

411

412 *Insularity and isolation promoting morphological divergence*

413 Whatever the dynamics of colonization, the insular conditions promoted a pronounced
414 morphological divergence in tooth morphology. This occurred in a relatively short evolutionary time
415 span, since the first appearance of *Acomys* in Crete occurred during the Hellenistic period, ~2000
416 years ago (Payne, 1995). The evolution on Cyprus is even more rapid. Since no *Acomys* fossil has
417 been recovered so far from the prehistoric record (Vigne, 1999; Horwitz et al., 2004), the species is
418 thought to have been introduced to this island during the last 1000 years. In that time span, the
419 molar morphology evolved markedly, exemplifying the acceleration of morphological evolution on
420 islands (Millien, 2006). Morphological divergence occurred as well in the isolated but not insular
421 population of Cilicia, although to a lesser degree. This suggests that random drift in isolated, small
422 populations fostered rapid divergence in Crete, Cyprus and Cilicia, and that adaptation to local insular
423 conditions has further drove molar evolution in the two insular populations, as shown for the house

424 mouse (Ledevin et al., 2016). In addition, hybridization between the populations of Crete might have
425 contributed to the diversity of molar shape on this island. Hybrid morphologies are not necessarily
426 intermediate between those of the parents, but can display transgressive phenotypes (Renaud et al.,
427 2017a). In Central Crete, molars frequently display an elongated forepart up to the occurrence of a
428 small, additional cusplet in front of the t2 cusp. A similar phenotype was found in hybrid house mice
429 (Renaud et al., 2017a), and in several insular house mouse populations (Renaud et al., 2011; Renaud
430 et al., 2018). The evolution of similar dental phenotypes in distant rodent groups might be due to
431 shared developmental processes favouring not only convergent evolution within true murines
432 (Hayden et al., 2020), but also between murines and the murine-like molar of *Acomys*.

433

434 *Heterogeneity in molar size: a partial decoupling from body size*

435 Evolution of size is a well-known characteristic of insular populations (Lomolino, 1985, 2005). Small
436 mammals are expected to increase in body size, due to a combination of factors including decrease
437 of interspecific competition and predation pressure, and increase of intraspecific competition
438 (Lomolino, 1985, 2005). Because molar size is considered to be a good proxy of body size at a broad
439 taxonomic scale (Gingerich et al., 1982), increase in molar size could be expected as well.

440 Regarding body size, *Acomys* indeed displayed a large body size on Cyprus, but within Crete, body
441 size varied considerably among populations (Fig. 4A). This may be related to different age structure
442 of the populations: sampling at different periods may lead to an overrepresentation of young or old
443 animals, skewing the size distribution towards small or large body size (Renaud et al., 2017b). Such a
444 confounding factor may be especially important in a species in which neonates already display hair
445 and open eyes, and can therefore rapidly leave the nest. Age structure may also contribute to the
446 large body size observed for most specimens from Cairo, that are mentioned to have been
447 maintained in captivity for some time.

448 Estimating the degree of insular size increase is all the more difficult, due to the lack of sufficient data
449 for African spiny mice. Nevertheless, among Eastern Mediterranean spiny mice, the continental
450 Cilician *Acomys* displayed the smallest body size. Differences between Cypriot and Cretan mice may
451 be related to local differences in competition and predation. For instance, the least weasel (*Mustela*
452 *nivalis*) has been intentionally introduced by Phoenicians and Greeks on the Mediterranean islands,
453 with the aim of controlling commensal rodents following their stowaway introduction (Rodrigues et
454 al., 2017). The weasels introduced in antiquity (Lehmann & Nobis, 1979), however went extinct in
455 Cyprus (Rodrigues et al., 2017), further relieving mammalian predation pressure on Cypriot *Acomys*.

456 In contrast, molar size varied greatly among Crete, Cyprus and Cilicia, and even within Crete. This
457 apparently surprising uncoupling between molar and body size is due to the fact that even if molar
458 size is correlated to body size at a broad taxonomic scale, it is not necessarily the case at a population
459 level, because the first molar erupts early after birth and is therefore not affected by subsequent
460 growth (Renaud et al., 2017b). The most obvious decoupling between body and molar size regards
461 the populations from Western Crete and Cairo, being of relatively large body size but extremely small
462 molar size (Fig. 4C). Similar uncoupling is not uncommon and has been observed for instance in wood
463 mice (*Apodemus sylvaticus*) from Ibiza (Renaud & Michaux, 2007).

464 The large variation in molar size may echo an under-evaluated variation in the African continent, as
465 suggested by the large variation in tooth size observed in Khartoum, Sudan (Fig. 4B). It could also
466 have an adaptive component, for instance related to a polarity in rainfall in Crete, western regions
467 receiving more rainfall and thus hosting different ecosystems. Indeed, larger tooth size
468 (“macrodonty”) has been proposed to occur in some insular small mammals, as a response to diet
469 shifts (Vigne et al., 1993). Large teeth with a massive outline, as those of Eastern Crete, might be
470 favoured in dry habitats, in order to process more efficiently hard food, resistant to comminution
471 (Renaud et al., 2005).

472

473 **Conclusion**

474 Genetic, chromosomal and morphometric results were congruent to underline a strong geographic
475 structure in Eastern Mediterranean spiny mice (*Acomys cahirinus* sensu lato) and especially within
476 Crete. A complex history of multiple introductions is probably responsible for this structure. Insular
477 isolation coupled with habitat shift must have further promoted a pronounced and rapid
478 morphological evolution in molar size and shape on Crete and Cyprus.

479 As a consequence, the species *A. nesiotus* (Cyprus), *A. cilicicus* (Cilicia, Turkey) and *A. minous* (Crete)
480 are clearly nested within *A. cahirinus* (group cah9 in (Aghová et al., 2019)) and are only defined by
481 their geographic distribution. Due to their isolated distribution and morphological characteristics,
482 “*nesiotus*” and “*cilicicus*” names may be maintained for describing subspecific evolutionary units. *A.*
483 *minous* encompasses populations with different haplotypic composition and morphometric
484 characteristics, and therefore it does not correspond to a homogeneous evolutionary unit. A more
485 thorough sampling of the African continent, as well as further genetic data including nuclear genes,
486 would be required for a better understanding of the complex history of translocation and evolution
487 in isolation that led to the amazing morphological diversity in modern Cretan and more generally,
488 Eastern Mediterranean spiny mice.

489

490 **Acknowledgements**

491 We thank the three anonymous reviewers for their constructive comments. The sampling in Turkey
 492 benefited from a funding by the Zonguldak Bulent Ecevit University project (no 2012-10-06-10) to
 493 Mustafa Sözen and Ferhat Matur.

494

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- 649

650 **Tables**

651

Area	Region	Locality	Code	N UM1	N Dloop	New accession numbers	
Crete	West (W)	Chania – Akrotirio	CHA	5	1	MT001851	
		Lefka Ori	LEFKO	5	1	MT001845	
		Chania – Souda	SOU	3	1	MT001848	
		Hills facing Elafonisos island				1	
			Kournas Lake			1	
	Central (C)	Almyros Gorge, Linoperamata	LINO	6	1	MT001852	
		Kokkini Hani	KOKH	8	1	MT001844	
		Stalida Mochos	STAL	3	1	MT001846	
		Piskopiano	PISKO	5	2	MT001847 MT043301	
	East (E)	Towards Kokkini Chani	PKOK	20	1	MT001853	
		Siteia	SIT	6	2	MT001849 MT001850	
		Vai				2	
	Cyprus	Cap Greco	CYP	6	5	MT001854-58	
Agirdag					1		
Cinarli					1		
Zafer Burnu, Cap Andreas					1		
Turkey	Cilicia (CIL)	Narlıkuyu	NAR	8	6	MT001830-35	
		Ayaş	AYA	4	3	MT001836-38	
		Karaahmetli, Hüseyinler, Kumrukuyu	KAR	7	5	MT001839-43	
		Silifke				2	
		NA				1	
Libya		Mts. Akakus			1		
Egypt	Cairo	CAIRO	8		2		
	Assouan				2		
Sahara	Sahara	Sudan (Kharthoum)	SAH	3			
		Chad (Yogoum)	SAH	1			
		Chad (Tibesti plateau)				1	
Fossil Crete	Central Crete	Kommos	Fos- KOM	3			

652

653 **Table 1.** Sampling for morphometric and genetic analyses. Region, area within region, locality of
654 trapping and its code are provided. N UM1: number of first upper molars included in the analysis. N
655 Dloop: number of Dloop sequences in the present study. In bold the number of newly acquired
656 sequences.

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Method	Variable(s)	Region	Sex	Interaction
ANOVA	HBL	< 0.0001	0.8050	0.9425
	UM1 CS	< 0.0001	0.1679	0.7630
ProcANOVA	UM1 Shape	< 0.0001	0.0133	0.0028

661

662 **Table 2.** Sexual dimorphism in molar size and shape, taking into account the regions. Probabilities of
663 ANOVA are given for size univariate parameters (HBL: head + body length; UM1 CS: first upper molar
664 centroid size) and of Procrustes ANOVA for shape data (aligned coordinates).

665

666 **Figure Captions**

667 **Figure 1.** Sampling localities and examples of first upper molars (UM1) in the various populations of
 668 *Acomys* considered in this study. All belong to the *cahirinus s.l.* group. Spiny mice from the island of
 669 Crete are designated as *A. minous*, those from the island of Cyprus as *A. nesiotetes* and those confined
 670 to a small area in Cilicia (Asia Minor, Turkey) as *A. cilicicus*. Nomenclature of the cusps is indicated on
 671 one molar tooth of *A. cilicicus*. The boxes indicate the color code of the areas in figures for
 672 morphometrics. For abbreviations of some localities (LINO, STAL, PISKO) see Table 1. All teeth to the
 673 same scale (scale bar bottom right).

674

675 **Figure 2.** Bayesian phylogeny of D-loop haplotypes of *Acomys cahirinus s.l.*. Posterior probability and
 676 bootstrap support are indicated for each node. “ – ” indicates that the node is not supported in the
 677 phylogeny reconstructed with PhyML.

678

679 **Figure 3.** Network and geographic distribution of the D-loop haplotypes. A. Median-joining haplotype
 680 network. B. Geographic repartition of the haplotypes. Each haplotype is identified by the same color
 681 code on both figures.

682

683 **Figure 4.** Size variation of *Acomys cahirinus s.l.* A. Body size (Head + Body Length) in the modern
 684 populations from Crete, Cyprus, Turkey, Cairo and Sahara. B. Size of the first upper molar (UM1) in the
 685 same populations; fossils from Kommos (Iron Age, Crete) are also included. C. Relationship between
 686 body and molar size. The Chadian specimen among the Sahara sample is indicated by “Chad” or “C”.
 687 The arrow with “No UM3” points to a young specimen without erupted third molar.

688

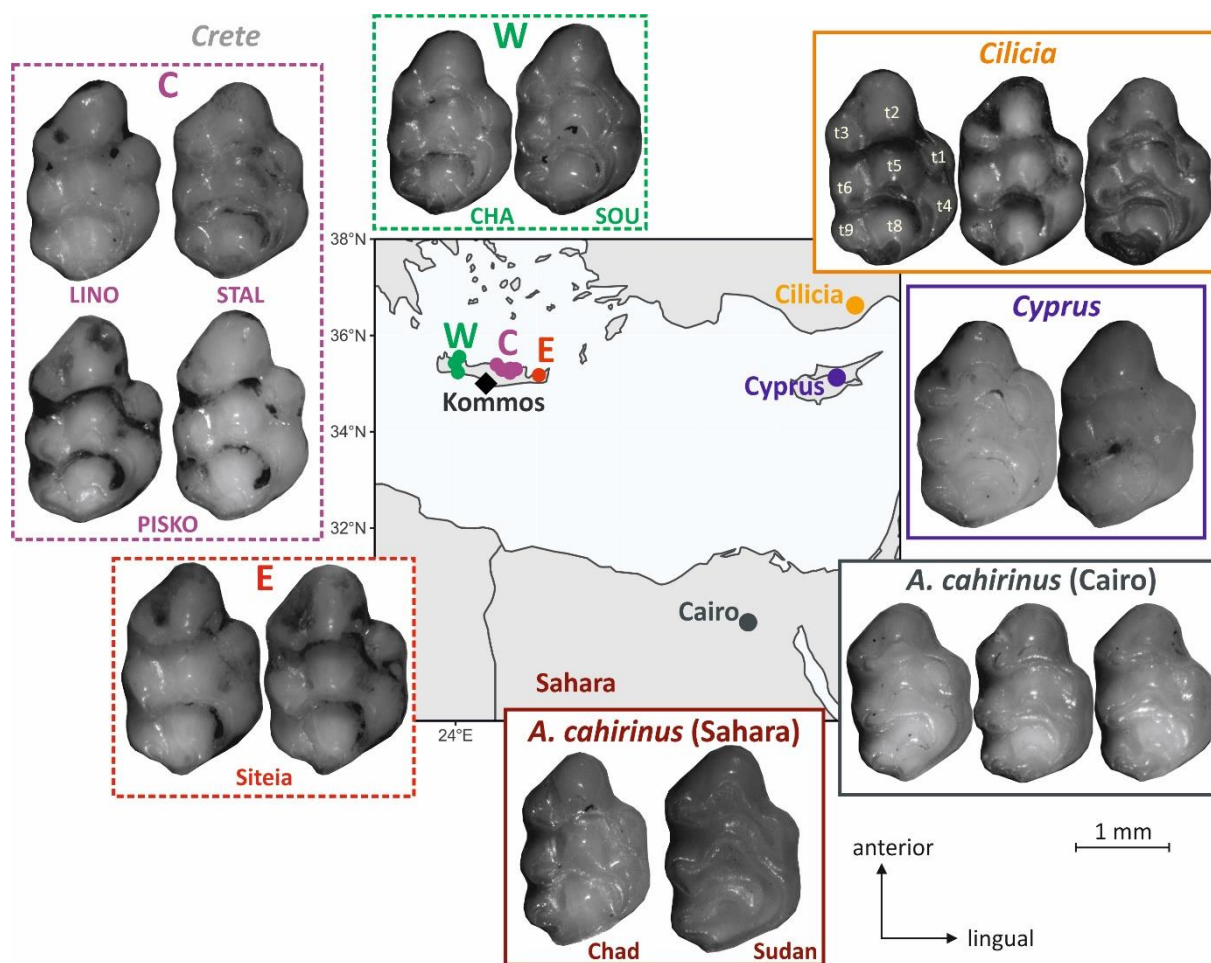
689 **Figure 5.** Shape differentiation of the first upper molar between the different populations of *Acomys*
 690 *cahirinus s.l.*, including the fossil teeth from Kommos (Iron Age, Crete). A. Morphospace defined by the
 691 first two axes of a PCA on the aligned coordinates of the points delineating the outline of the first upper
 692 molar. Each dot corresponds to a tooth. “C” indicates the Chadian specimen in the Sahara sample. B,
 693 C: Deformation along the PC axes. Arrows point from the shape corresponding to the minimal score,
 694 to the shape corresponding to the maximal score (B: PC1; C: PC2). D. Means of the geographic group
 695 in the morphospace defined by the first two axes of a bgPCA. Distance between grid bars: $d=0.02$.
 696 Abbreviations: cf. Table 1.

697 **Figure 6.** Allometric shape differentiation of the first upper molar. A. Allometric shape variation. Size
698 is estimated by the centroid size of the UM1; shape by the Common Allometric Component (CAC). "C"
699 indicates the Chadian specimen in the Sahara sample. B. Allometric deformation. Arrows point from
700 the shape corresponding to the minimal centroid size, to the shape corresponding to the maximal
701 centroid size. C. Means of the geographic group in the morphospace defined by the first two axes of a
702 bgPCA on the residuals of a regression of the aligned coordinates vs. size. Distance between grid bars:
703 $d=0.02$.

704

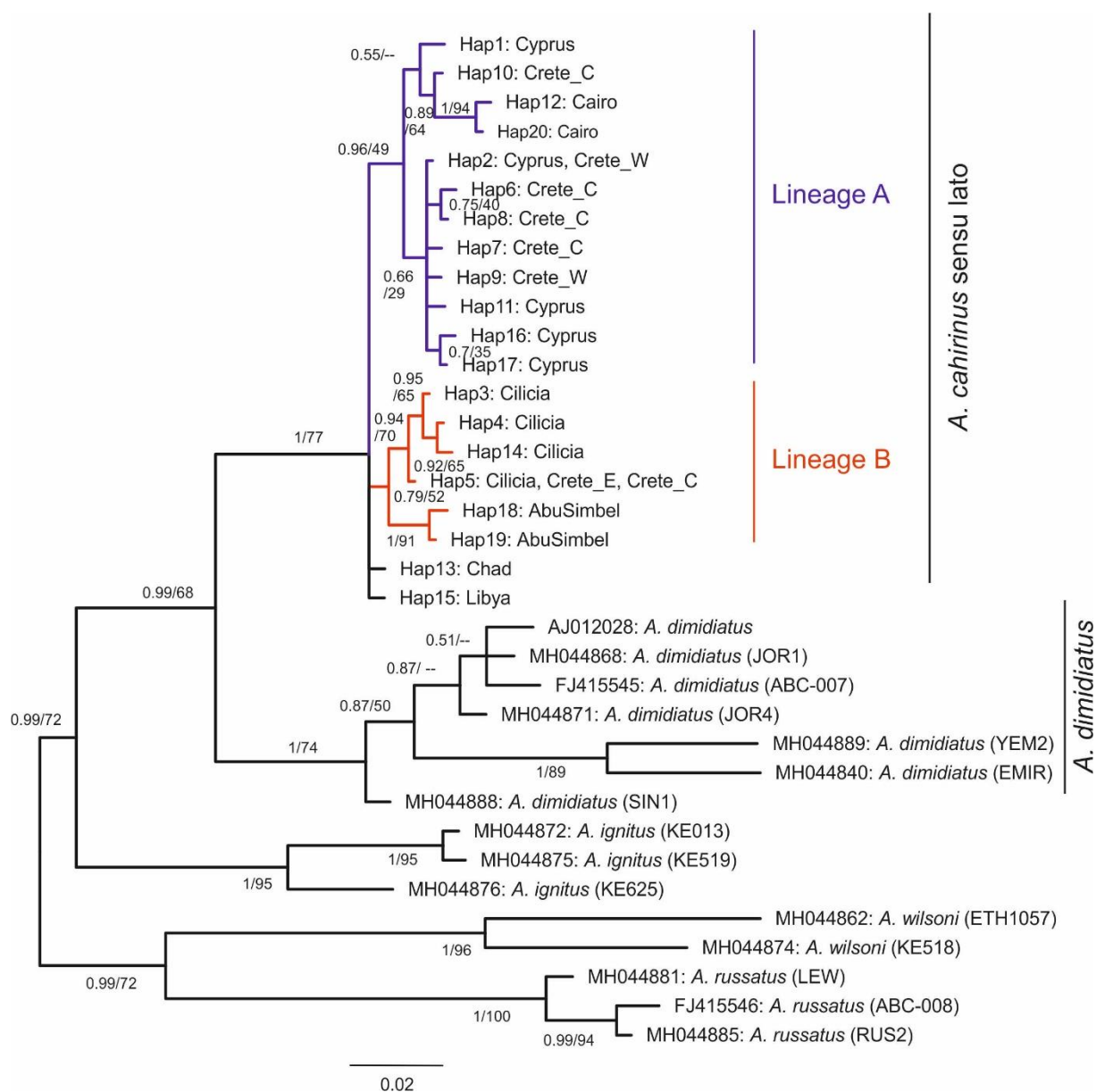
705 **Figure 7.** Karyotypic variation of *Acomys cahirinus* s.l. in the Eastern Mediterranean area. The pie charts
706 indicate the proportion of the $2n$ numbers in the different populations. Data from Giagia-
707 Athanasopoulou et al. (2011).

708



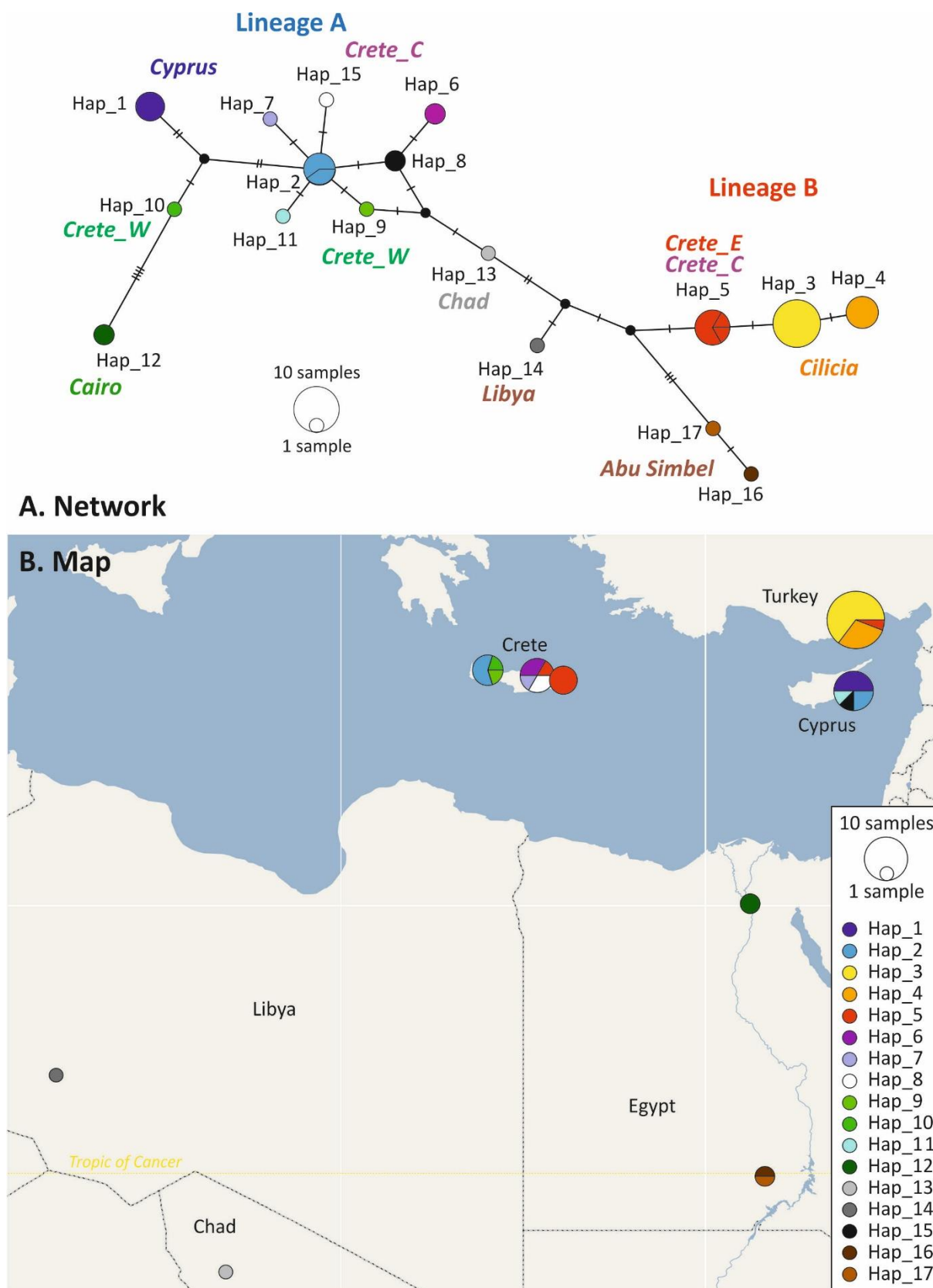
709

710 **Figure 1.** Sampling localities and examples of first upper molars (UM1) in the various populations of
 711 *Acomys* considered in this study. All belong to the *cahirinus s.l.* group. Spiny mice from the island of
 712 Crete are designated as *A. minous*, those from the island of Cyprus as *A. nesiotetes* and those confined
 713 to a small area in Cilicia (Asia Minor, Turkey) as *A. cilicicus*. Nomenclature of the cusps is indicated on
 714 one molar tooth of *A. cilicicus*. The boxes indicate the color code of the areas in figures for
 715 morphometrics. For abbreviations of some localities (LINO, STAL, PISKO) see Table 1. All teeth to the
 716 same scale (scale bar bottom right).



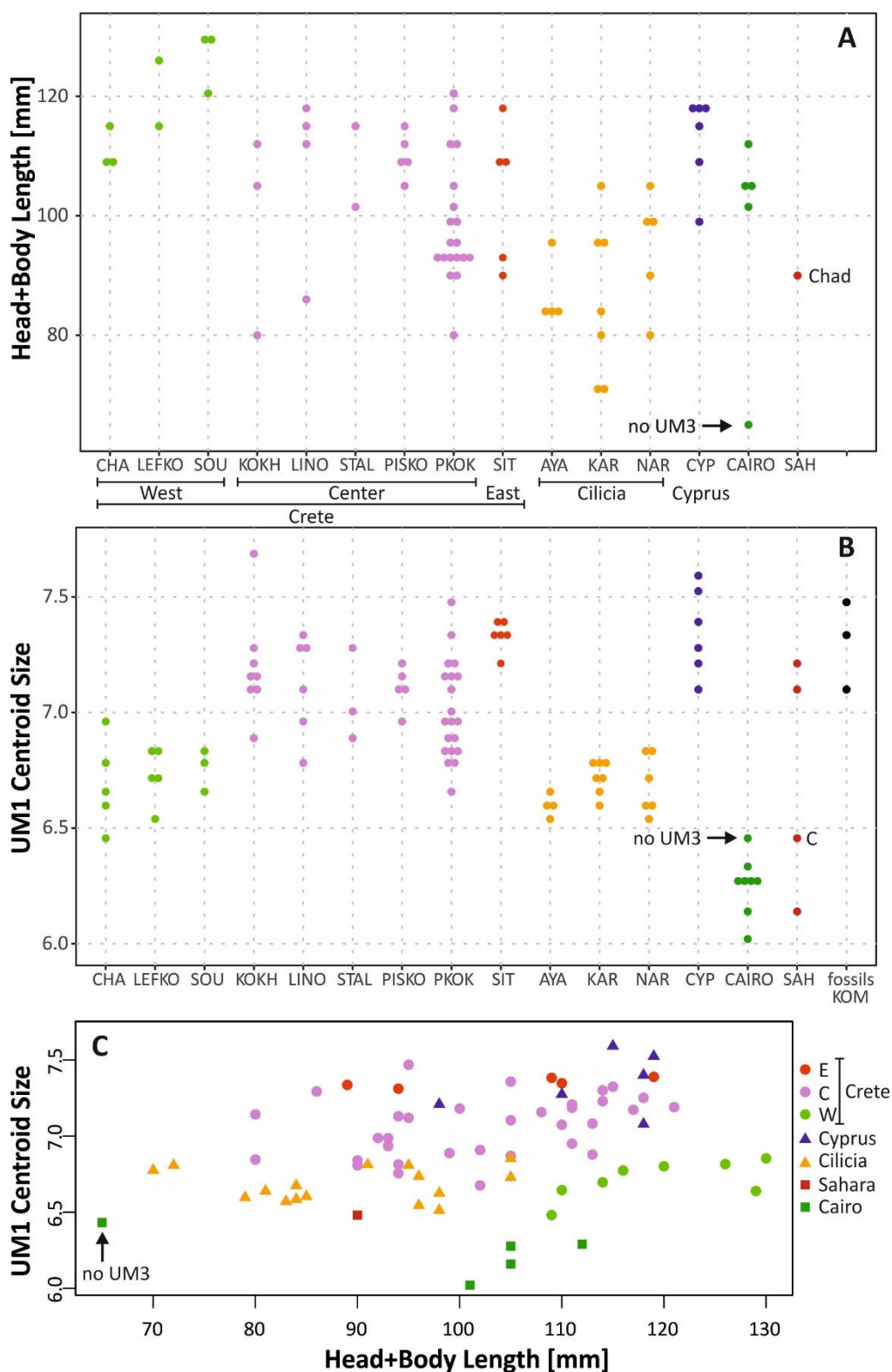
717

718 **Figure 2.** Bayesian phylogeny of D-loop haplotypes of *Acomys cahirinus sensu lato* (i.e. including *A.*
 719 *minous*, *A. nesiotus* and *A. cilicicus*). Posterior probability and bootstrap support are indicated for
 720 each node. “ -- ” indicates that the node is not supported in the phylogeny reconstructed with PhyML.



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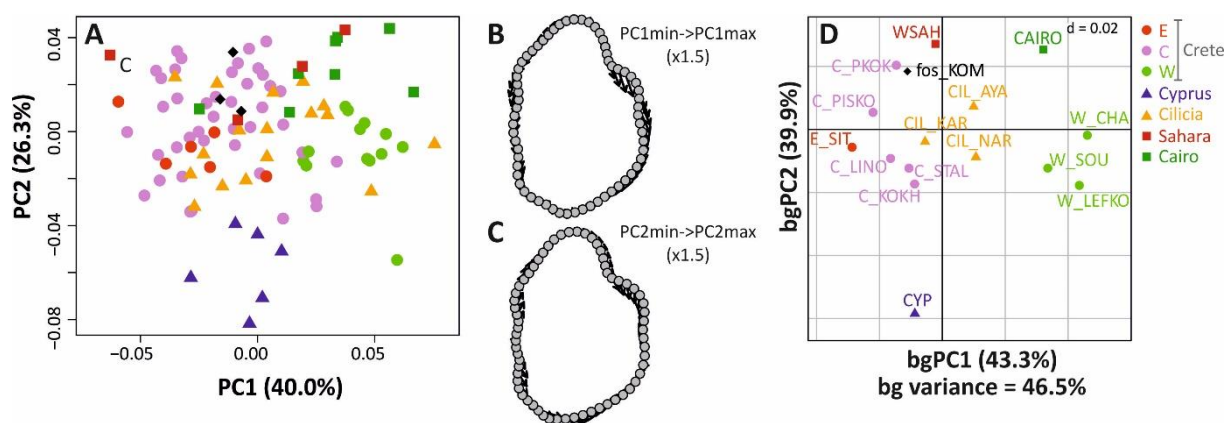
722 **Figure 3.** Network and geographic distribution of the D-loop haplotypes. A. Median-joining haplotype
 723 network. B. Geographic repartition of the haplotypes. Each haplotype is identified by the same color
 724 code on both figures.



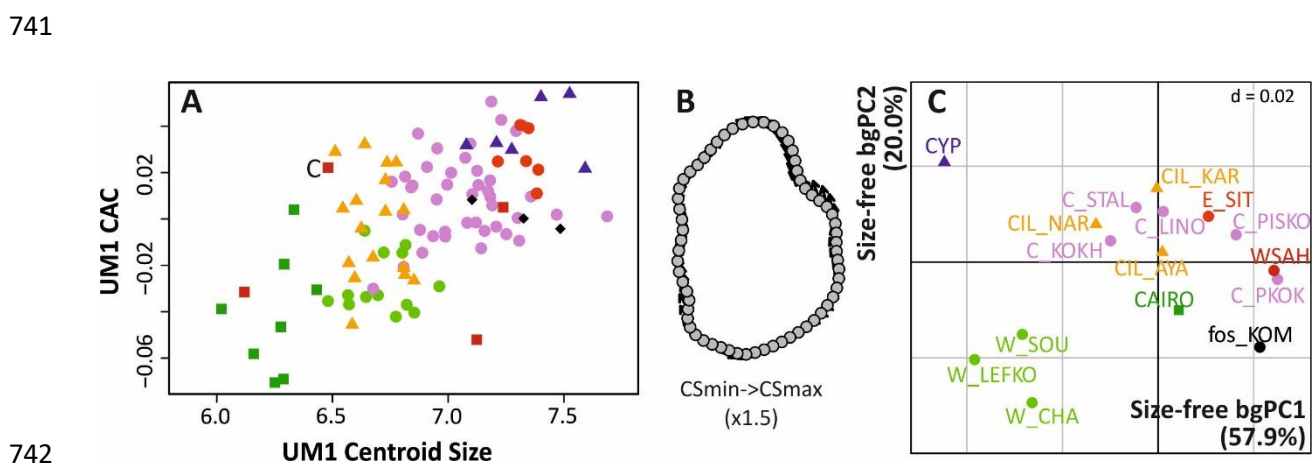
725

726 **Figure 4.** Size variation of *Acomys cahirinus* s.l. A. Body size (Head + Body Length) in the modern
 727 populations from Crete, Cyprus, Turkey, Cairo and Sahara. B. Size of the first upper molar (UM1) in the
 728 same populations; fossils from Kommos (Iron Age, Crete) are also included. C. Relationship between
 729 body and molar size. The Chadian specimen among the Sahara sample is indicated by “Chad” or “C”.
 730 The arrow with “No UM3” points to a young specimen without erupted third molar.

731



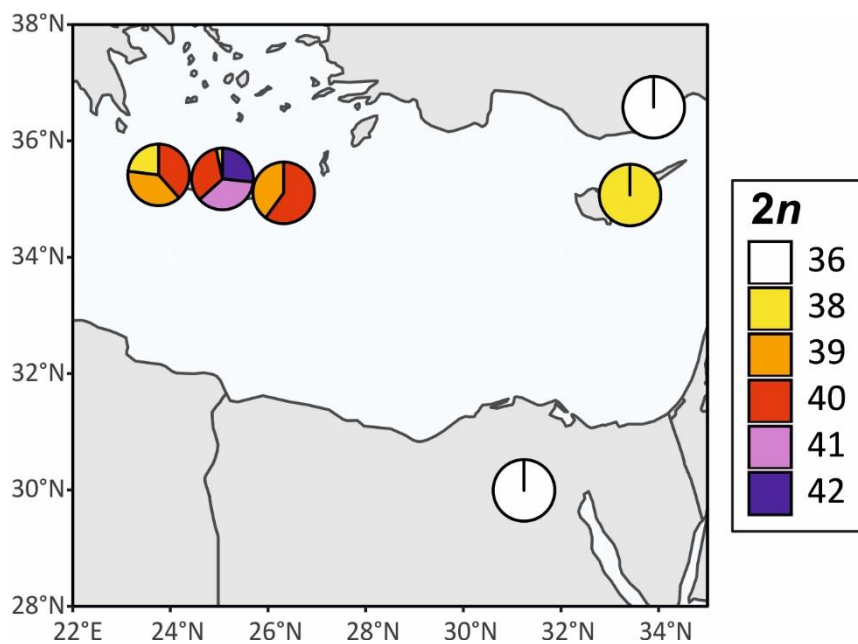
732
 733 **Figure 5.** Shape differentiation of the first upper molar between the different populations of *Acomys*
 734 *cahirinus* s.l., including the fossil teeth from Kommos (Iron Age, Crete). A. Morphospace defined by the
 735 first two axes of a PCA on the aligned coordinates of the points delineating the outline of the first upper
 736 molar. Each dot corresponds to a tooth. “C” indicates the Chadian specimen among the Sahara sample.
 737 B, C: Deformation along the PC axes. Arrows point from the shape corresponding to the minimal score,
 738 to the shape corresponding to the maximal score (B: PC1; C: PC2). D. Means of the geographic group
 739 in the morphospace defined by the first two axes of a bgPCA. Distance between grid bars: $d=0.02$.
 740 Abbreviations: cf. Table 1.



742
 743 **Figure 6.** Allometric shape differentiation of the first upper molar shape. A. Allometric shape variation.
 744 Size is estimated by the centroid size of the UM1; shape by the Common Allometric Component (CAC).
 745 “C” indicates the Chadian specimen among the Sahara sample. B. Allometric deformation. Arrows
 746 point from the shape corresponding to the minimal centroid size, to the shape corresponding to the
 747 maximal centroid size. C. Means of the geographic group in the morphospace defined by the first two
 748 axes of a bgPCA on the residuals of a regression of the aligned coordinates vs. size. Distance between
 749 grid bars: $d=0.02$.

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754 **Figure 7.** Karyotypic variation of *Acomys cahirinus* s.l. in the Eastern Mediterranean area. The pie charts
 755 indicate the proportion of the $2n$ numbers in the different populations. Data from Giagia-
 756 Athanasopoulou et al. (2011).

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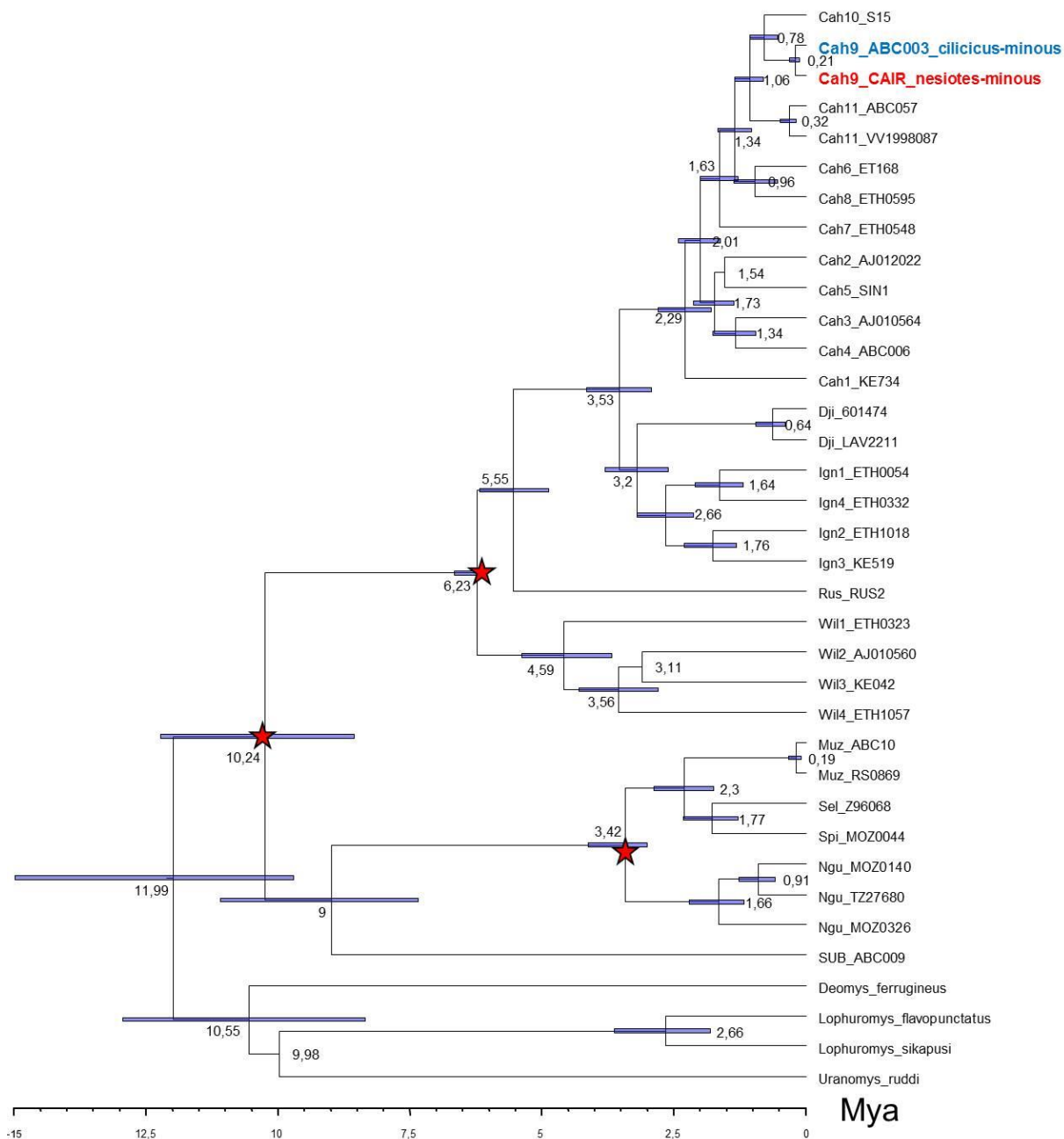
761

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763 **Supplementary Informations**

764

765 **Supplementary Figure 1.** Chronogram obtained with BEAST. Values on the nodes represent medians
 766 of estimated divergence date, and the horizontal bars show 95% highest posterior density of these
 767 estimates. Red stars indicate the positions of three fossil constrains used for the calibration of
 768 molecular clock.



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772 **Supplementary Table 1.** Accessions of the sequences used in the dating analysis. Lineages and
 773 groups defined as in Aghova et al. (2019).
 774

Genus	Species	Lineage	Group	Cytb	D-loop	IRBP	RAG1
<i>Acomys</i>	<i>cineraceus</i>	<i>Cah1</i>	<i>cahirinus</i>	MH044976	MH044879	MH044739	MH044833
<i>Acomys</i>	sp. 2	<i>Cah2</i>	<i>cahirinus</i>	AJ012022			
<i>Acomys</i>	sp. 1	<i>Cah3</i>	<i>cahirinus</i>	AJ010564			
<i>Acomys</i>	<i>johannis</i>	<i>Cah4</i>	<i>cahirinus</i>	FJ415483	FJ415544	MH044740	MH044818
<i>Acomys</i>	<i>dimidiatus</i>	<i>Cah5</i>	<i>cahirinus</i>	MH044985	MH044888	MH044767	MH044829
<i>Acomys</i>	<i>mullah</i>	<i>Cah6</i>	<i>cahirinus</i>	MH044992	MH044845	MH044742	MH044834
<i>Acomys</i>	sp. B	<i>Cah7</i>	<i>cahirinus</i>	KX290493	MH044857	MH044764	MH044826
<i>Acomys</i>	sp. A	<i>Cah8</i>	<i>cahirinus</i>	MH045013	MH044858	MH044761	MH044836
<i>Acomys</i>	<i>cahirinus</i>	<i>Cah9_Lineage A</i>	<i>cahirinus</i>	MH045014	MH044837	MH044772	MH044832
<i>Acomys</i>	<i>cahirinus</i>	<i>Cah9_Lineage B</i>	<i>cahirinus</i>	FJ415480	FJ415541	MH044773	MH044831
<i>Acomys</i>	sp. Cah10	<i>Cah10</i>	<i>cahirinus</i>	MH045016		MH044753	MH044817
<i>Acomys</i>	<i>chudeaui</i>	<i>Cah11</i>	<i>cahirinus</i>	FJ415534	FJ415595		
<i>Acomys</i>	<i>chudeaui</i>	<i>Cah11</i>	<i>cahirinus</i>	MH045006		MH044755	MH044822
<i>Acomys</i>	<i>louisae</i>	<i>Dji</i>	<i>cahirinus</i>	MH044903			MH044805
<i>Acomys</i>	<i>louisae</i>	<i>Dji</i>	<i>cahirinus</i>	MH044900		MH044735	
<i>Acomys</i>	sp. C	<i>Ign1</i>	<i>cahirinus</i>	MH045020	MH044850	MH044747	MH044813
<i>Acomys</i>	sp. Ign2	<i>Ign2</i>	<i>cahirinus</i>	MH044971	MH044859	MH044743	MH044807
<i>Acomys</i>	<i>ignitus</i>	<i>Ign3</i>	<i>cahirinus</i>	MH044968	MH044875	MH044756	MH044812
<i>Acomys</i>	<i>kempi</i>	<i>Ign4</i>	<i>cahirinus</i>	MH045033	MH044855	MH044744	MH044809
<i>Acomys</i>	<i>russatus</i>	<i>Rus</i>	<i>russatus</i>	MH044905	MH044885	MH044733	MH044788
<i>Acomys</i>	<i>muzei</i>	<i>Muz</i>	<i>spinosissimus</i>	FJ415487	FJ415548		
<i>Acomys</i>	<i>muzei</i>	<i>Muz</i>	<i>spinosissimus</i>	MG434400		MG434355	
<i>Acomys</i>	<i>ngurui</i>	<i>Ngu</i>	<i>spinosissimus</i>	MG434388		MG434353	
<i>Acomys</i>	<i>ngurui</i>	<i>Ngu</i>	<i>spinosissimus</i>	MG434396		MG434354	
<i>Acomys</i>	<i>ngurui</i>	<i>Ngu</i>	<i>spinosissimus</i>	MG434414		MG434358	
<i>Acomys</i>	<i>selousi</i>	<i>Sel</i>	<i>spinosissimus</i>	Z96068			
<i>Acomys</i>	<i>spinosissimus</i>	<i>Spi</i>	<i>spinosissimus</i>	MG434385		MG434352	
<i>Acomys</i>	<i>subspinosus</i>	<i>Sub</i>	<i>subspinosus</i>	FJ415486	FJ415547	MH044731	MH044787
<i>Acomys</i>	<i>percivali</i>	<i>Wil1</i>	<i>wilsoni</i>	MH044950	MH044854	MH044783	MH044792
<i>Acomys</i>	sp. 'Magadi'	<i>Wil2</i>	<i>wilsoni</i>	AJ010560			
<i>Acomys</i>	aff. <i>percivali</i>	<i>Wil3</i>	<i>wilsoni</i>	MH044911	MH044873	MH044780	MH044795
<i>Acomys</i>	<i>wilsoni</i>	<i>Wil4</i>	<i>wilsoni</i>	MH044912	MH044862	MH044774	MH044797
<i>Deomys</i>	<i>ferrugineus</i>		OUTGROUP	FJ415478	FJ415539	AY326084	
<i>Lophuromys</i>	<i>flavopunctatus</i>		OUTGROUP	EU349754		AY326091	AY294950
<i>Lophuromys</i>	<i>sikapusi</i>		OUTGROUP	AJ012023		AJ698899	KC953515
<i>Uranomys</i>	<i>ruddi</i>		OUTGROUP	HM635858	FJ415540	EU360812	DQ023454

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Subset	Best Model	Sites number	Partition names
1	TRN+I+G	335	Cytb_pos1
2	HKY+I+G	335	Cytb_pos2
3	HKY+G	335	Cytb_pos3
4	K80+I	1319	IRBP_pos3, RAG1_pos1, RAG1_pos2, IRBP_pos1
5	HKY+G	659	IRBP_pos2, RAG1_pos3
6	HKY+I+G	526	Dloop

778

779 **Supplementary Table 2.** Substitution models used in the BEAST analysis.

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