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1 **Phylogenetic relationships between genera *Dorcadion*, *Lamia*, *Morimus*, *Herophila* and**
2 **some other Lamiinae (Coleoptera: Cerambycidae) based on chromosome and CO1 gene**
3 **sequence comparison**

4

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13

14 **Key Words:** Lamiinae, CO1 sequencing, karyotype, phylogeny, monophyly.

15

16 **Short title:** Monophyly of genera *Dorcadion*, *Lamia*, *Herophila* and *Morimus*

17

18

19 **Summary**

20 A dual molecular and cytogenetic study was performed with the aim to improve the
21 controversial systematic classification of some species of Lamiinae (Coleoptera:
22 Cerambycidae). The karyotypes of species belonging to genera *Morimus*, *Herophila*,
23 *Dorcadion*, *Neodorcadion* and *Lamia* share a number of characters, which differentiate them
24 from other species, belonging to genera *Phytoecia*, *Parmena* and *Monochamus*. The
25 karyotypes of the last 3 species comprise 20 chromosomes, mostly metacentric or sub-

26 metacentric, as in the presumed Cerambycidae ancestors. The karyotypes of the former
27 species share many characters derived from the Lamiinae ancestors by a number of
28 chromosome fissions and inversions indicating their monophyly. Comparisons of the CO1
29 gene sequence also show the monophyly of *Morimus*, *Lamia*, *Herophila* and *Dorcadion* and
30 their distant relationship with others. These convergent results allow us to propose a
31 phylogenetic classification of these genera, which places the monospecific genus *Lamia* close
32 to *Dorcadion*, clearly separates *Dorcadion* and *Neodorcadion* and places *Herophila* closer to
33 *Morimus* than to *Dorcadion/Lamia*. The genus *Morimus* is the most derived. CO1 mutations
34 loosely separate the forms *M. asper* and *M. funereus*, which have similar karyotypes and
35 behaviour and copulate in captivity. The form *M. ganglebaueri* may have a *funereus* X *asper*
36 hybrid origin.

37

38 **Introduction**

39 Lamiinae constitute the largest sub-family of Cerambycidae, one of the largest families of
40 Coleoptera. which form the largest order of the animal kingdom. Thus, with more than 21,000
41 species, Lamiinae represent one of the largest taxonomic animal entity. Latreille created this
42 sub-family in 1825 and considered *Lamia textor* Linnaeus, 1758 for its type species. The
43 genus *Lamia* remained mono-specific with *L. textor* for its single species and was included, in
44 the tribe Lamiini Latreille, 1825. Other tribes, such as Dorcadionini Latreille, 1825, Agniini
45 Thomson, 1864, Monochamini Thomson, 1860 and Phrissomini Thomson, 1860 were further
46 created, and shared variously some of the species initially included in the Lamiini. Today, the
47 general trends are either to transfer species belonging to Lamiini to Monochamini or include
48 all Monochamini into Lamiini, but these changes are principally based upon the analysis of
49 phenotypic characters (Bousquet et al. 2009, Lobl and Smetana, 2010, Monné et al., 2017).
50 Beside *Lamia*, the genus *Morimus* Brullé, 1832 is composed of a small number of Palearctic

51 species/sub-species, which have alternatively been classified into Lamiini or Phrissomini.
52 Phrissomini were isolated from Lamiini to group apterous species but the present trend is to
53 consider this distinction not valid. There is no more unanimity about the distribution of
54 species into these tribes. In Europe, the number of recognized species of *Morimus* varies from
55 1 to 4, depending on the elevation of three taxons (*ganglbaueri*, Reitter, 1884, *verecundus*,
56 Falderman, 1836, and *funereus*, Mulsant, 1862) to the rank of species, or their classification as
57 simple sub-species of *M. asper* Sulzer, 1776. About 10 other species occur in oriental regions.
58 For the sake of simplification, we will arbitrarily call the taxa studied here *M. asper*, *M.*
59 *ganglbaueri* and *M. funereus*, without presumption of the validity of their species status. The
60 genus *Morimus* shares a number of morphological characters with the genus *Herophila*,
61 Mulsant, 1863. Both are often included in the tribe Phrissomini. Their morphology recalls that
62 of *Lamia textor* Linnaeus, 1758 and also that of species of *Dorcadion* Dalman, 1817
63 generally classified into Dorcadionini. Very few cytogenetic studies were performed (Smith
64 and Virkki, 1978, Dutrillaux and Dutrillaux, 2011, Okutaner et al., 2011) and there is neither
65 conclusions about the species status within genera, nor the phylogenetic relationships between
66 genera. In a recent study of CO1 and ITS2 genes, developed on *M. asper* and *M. funereus*
67 specimens from Italy, Croatia, Montenegro Slovenia, Turkey and Peloponnese, Solano et al.
68 (2013) concluded that their genetic distance is small and compatible with the presence of a
69 single species. The aim of our work was to provide genetic criteria for improving
70 phylogenetic relationships and classification of genera *Morimus*, *Lamia*, *Herophila* and
71 *Dorcadion* by the study and comparison of both their chromosomes and their CO1 gene
72 sequence. For genus *Morimus* specimens from 2 unexplored regions, France and continental
73 Greece were selected. Two of the 3 species of *Herophila* could also be studied. For genera
74 *Dorcadion/Neodorcadion*, the systematic classification is so conflicting that we restricted our
75 report on a few consensual species amongst the many species we studied. Species of 3 other

76 genera of Lamiinae: *Parmena* (Parmenini), *Monochamus* Dejean, 1821 (Monochamini Gistel,
77 1848) and *Phytoecia* Mulsant, 1832 (Phytoeciini Mulsant, 1839); are used as outgroups.

78

79 **Material and Methods**

80 **Animals**

81 *Morimus asper*: male specimens from France, both sides of the Verdon river, near La Palud-
82 sur-Verdon (43°46'48''N, 06°20'30''E) and near Aiguines (43°46'33''N, 06°14'37''E), and
83 Greece, Tripi, near Sparti (37°04'10''N, 22°16'20''E, Fig. 1 a) Peloponisos, near Metsovo
84 (39°46'09N, 21°10'55''E, Fig. 1f) West Macedonia/Epirus border, Mount Parnassus
85 (38°33'09''N, 22°33'30''E, Fig. 1d) Central Greece.

86 *Morimus funereus*: male specimens from Greece, Thessaly: Mount Ossa (39°47'59''N,
87 22°41'13''E, Fig. 1h); Mount Olympus (40°05'08''N, 22°21'31'', Fig. 1k) and Mount Pelion,
88 near Volos (39°30'N, 22°57'E); West Macedonia: mount Pelister near Kastoria (40°31'N,
89 21°18'E, Fig. 1g); East Macedonia/Thrace bordure: Central Rodopi, Kariofito, near
90 Stavropouli (40°40'N, 22°56'E, Fig. 1j).

91 *Morimus ganglbaueri* Reitter, 1894: One male and one female specimens from Bourazani,
92 near Albanian border (40°03'13''N, 20°37'36''E, Fig. 1l), Epirus, Greece.

93 *Herophila tristis* Linnaeus, 1767: male specimens from Greece, near Lake Prespa (40°46'N,
94 21°06'E), West Macedonia and Bourazani (Fig. 1n, o).

95 *Herophila faimairei* Thomson, 1857: female specimens from Mount Parnassus (Fig. 1p).

96 *Lamia textor* Linnaeus, 1758: one female from the south of France (Fig. 1s).

97 *Dorcadion (Cribridorcadion) thessalicum* Pic, 1916: males from Meteora region (39°42'N,
98 21°37'E, Fig. 1t), Thessaly, Greece.

99 *Dorcadion (Cribridorcadion) equestre* Laxman, 1770: males from Ohrid region (41°07'N,
100 20°48'E, Fig. 1u), Republic of Macedonia.

101 *Dorcadion (Cribridorcadion) obenbergeri* Heyrovsky, 1940 from Vermio Mountains
 102 (40°37'26"N, 21°56'33"E, Fig. 1v), Macedonia, Greece.

103 *Dorcadion (Iberodorcadion) fuliginator* Linnaeus, 1758: males from Irun region (43°20'N,
 104 01°47'W, Fig. 1m), Spain.

105 *Neodorcadion bilineatum* Germar, 1824: males from Mount Pelion, Thessaly (39°26'N,
 106 23°02' E, Fig. 1q) and several other places in Greece.

107 *Neodorcadion exornatum*: males from Microderio (41°18'N, 26°01'E, Fig. 1r) Thraki,
 108 Greece.

109 *Parmena pubescens* Dalman, 1817: one female from Methoni (36°50'N, 21°40'E, Fig. 1x)
 110 Peloponisos, Greece.

111 *Monochamus sutor* Linnaeus, 1758: male from Stavroupoli region (41°12'N, 24°42'E, Fig.
 112 1y) Thraki, Greece.

113 *Phytoecia (Musaria) affinis* Harrer, 1784: male and female specimens from Mount Olympus
 114 (40°04'55"N, 22°20'55" E, Fig. 1w).

115

116 **Cytogenetic techniques**

117 Proliferating cells of three origins were obtained: testicles, mid gut and eggs.

118 *Testicular follicles.* Follicles were extracted from the abdominal cavity. Each follicle was
 119 immediately dropped into a 0.88 g/l KCl aqueous solution where it remained for 15 min at
 120 room temperature. After centrifugation, it was transferred into a micro-centrifuge tube (VWR
 121 International SAS, code 211-0033) containing a 0.55 g/l KCL solution, where it was squashed
 122 and suspended using a piston (VWR, code 045520) adapted to the tube. The volume of the
 123 supernatant was increased to 1.5 ml by addition of 0.55 KCl solution. After 10 min, the
 124 hypotonic solution was replaced by Carnoy 1 fixative where the cells were suspended and left
 125 for a minimum of 30min. After one change of fixative (all centrifugations 5 min at 800g), the

126 cells were spread on wet and cold slides. Giemsa staining, C-banding, NOR-staining were
127 performed as described (Angus, 1982, 1988, Dutrillaux et al. 2006, Howell and Black, 1980).

128 *Mid-gut cells*. Dissection was performed in 0.88 g/l KCl. About 0.3 cm long fragments of mid
129 gut were cleared out of their content and put in a watch-glass containing 0.88 KCl solution.
130 The fragments were dilacerated, one drop of colcemid solution was added and the mixture of
131 suspended cells and remaining fragments was left for one hour before being squashed and
132 treated as above, except that the KCl solution was replaced by fetal calf serum diluted in
133 distilled water (1 vol::2vol).

134 *Eggs*. The main difficulty is the choice of the best moment to obtain dividing cells. We
135 selected the period when the eggs are swelling. Eggs were cleaned up and put directly in a
136 micro-centrifuge tube containing either a 0.55 KCL or diluted serum solution. Then, they
137 were squashed and treated as described above.

138 All karyotypes and chromosome measurements were performed using the IKAROS device
139 (METASYSTEMS, Germany).

140

141 **Sequencing analysis of the mitochondrial COI gene**

142 Mitochondrial DNA was isolated from 34 individuals. A 525 bp segment at the 3' end of the
143 *COI* gene was amplified using the primers C1J 1718 (5'-
144 GGAGGATTTGGAGGTTGATTAGTTCC-3') and C1N 2191 (5'-
145 CCCGGTAAAATTAAAATATAAACTTC-3') (Simon *et al.*, 1994). PCR reactions (50 µl)
146 contained 200–500 ng DNA, 10× Taq buffer, 2 mM MgCl₂, 0.2 mM of each dNTP, 50
147 pmoles of each primer and 1 U Taq polymerase (Invitrogen, Carlsbad, CA, USA). The
148 cycling conditions consisted of an initial denaturation at 95°C for 5 min followed by 35 cycles
149 of denaturation at 95°C for 30 s, annealing at 52°C for 40 s and extension at 72°C for 1 min,
150 with a final extension at 72°C for 10 min. PCR products were purified using QIAquick PCR

151 purification kit (QIAGEN Cat. No. 28106, Valencia, CA, USA) and were sequenced directly
152 and bi-directionally by Macrogen Inc. Nucleotide sequences were aligned using ClustalX
153 (Larkin *et al.*, 2007).

154 For all haplotypes, base composition, nucleotide variation, polymorphic and parsimony
155 informative sites, the appropriate model of sequence evolution and phylogenetic associations
156 among lineages were assessed using MEGA version 5 (Tamura *et al.* 2011). According to BIC
157 scores (Bayesian Information Criterion) the GTR+G+I model [General Time Reversible
158 modeled by using a discrete Gamma distribution (+G) with 5 rate categories and by assuming
159 that a certain fraction of sites are evolutionarily invariable (+I)] was selected for subsequent
160 analyses and the construction of a neighbor-joining (NJ) tree. Parsimony and ML trees were
161 also constructed. Node support was assessed on the basis of 1000 bootstrap replicates. For all
162 trees, the sequence of *Aegosoma scabricorne* were used as outgroup.

163 A Bayesian analysis was also performed with MRBAYES version 3.1 (Huelsenbeck &
164 Ronquist, 2001), under the HKY85 model of sequence evolution. Depending on the data set,
165 random starting trees run for 2×10^6 to 8×10^6 generations were used, sampled every 100
166 generations. Burn-in frequency was set to the first 25% of the sampled trees.

167

168 **Results**

169

170 **Species identification.** All the specimens studied here were captured by in nature. We had no
171 difficulty for determining their specific or sub-specific status, which agreed with the known
172 localities of the species, except for the two specimens of *Morimus* from Bourazani (Epirus,
173 Greece). Their antennae are large and the surface of their elytrons is completely granular, as
174 in *M. asper asper*, whereas the elytra pubescence is pale grey and dark spotted, as in *M.*
175 *funereus*. These morphologic characters correspond to those described for *Morimus*

176 *ganglbaueri* Reitter, 1894. However, this species is known from Dalmatian coast (Bosnia,
177 Montenegro and Albania), but not from Greece. Thus, if our identification is correct, this
178 species occurs in Greece, at least in border zones with Albania. It may be also that these
179 specimens are hybrids between *M. asper* and *M. funereus*.

180 **Breeding assays.** *Morimus* imagines, fed with twigs of various species of trees, can be kept
181 alive for several months, i.e., from the month of May until March of the following year in our
182 experience. We kept couples of *M. funereus* and one couple formed by a male *M. asper asper*
183 and a female *M. funereus* captured in nature in Greece. They co-existed peacefully for months
184 with frequent ridings, and their behaviour was similar to that of other couples. Eggs were laid
185 5 months later, and first instar larvae could colonise a branch of cherry-wood from
186 Fontainebleau region (where genus *Morimus* does not occur). They were maintained at room
187 temperature and a single female imago was obtained on July. Its phenotype was typically that
188 of *M. funereus*, except that its dark spots were granulated, as in *M. ganglbaueri* and *M. asper*
189 *asper*, and not *M. funereus*. Unfortunately, we did not know whether or not the female,
190 captured in nature, was virgin. This makes uncertain the hybrid status of the descendant.

191

192

193 **Chromosomal data**

194 *Morimus asper*. Published data: none. The karyotype is composed of 22 autosomes, a sub-
195 metacentric X and a punctiform Y: 24,XY in the males and 24,XX in the females. Three
196 autosomes (1, 2 and 3) are almost metacentric and one autosome (4) is sub-metacentric, with a
197 largely heterochromatic short arm. All other autosomes are acrocentric, with very small
198 amounts of heterochromatin in their short arms (Fig. 2.1). This description prevails for
199 specimens of all geographic origins in which only few variations of juxta-centromeric (C-
200 banded) heterochromatin were observed. The most variable segment is the short arm of

201 chromosome 6. At the pachytene stage of meiosis, this arm is elongated, and deeply silver
202 stained indicating it harbours the Nucleolus Organizer Region (NOR). At the first meiotic
203 division, there are 11 autosomal bivalents and the sex bivalent has a parachute configuration:
204 $11+X_{yp}$.

205 *Morimus ganglbaueri*. Published data: none. The karyotype is fairly similar to that described
206 above: $24,XY$ and $24,XX$ in the male and female, respectively (Fig. 2.2). Heterochromatin is
207 slightly more intensely C-banded and may be present in intercalary position of some
208 chromosomes.

209 *Morimus funereus*. Published data: description of a disomy Y: $25,XY Y$ (Dutrillaux and
210 Dutrillaux, 2011). The karyotype, similar in specimens from the different localities, does not
211 look different from that of the previous taxa: $24,XY$ and $11+X_{yp}$ in the males. Intercalary
212 heterochromatin is occasionally present (chromosome 5, 7) (Fig. 2.3).

213 *Herophila tristis*. Published data: none. Its karyotype is also composed of 24 chromosomes
214 ($24,XY$ in the males), but compared to that of *Morimus* species, only 2 pairs of autosomes are
215 metacentric (Fig. 2.4). The 2 largest pairs of acrocentrics carry large and variable amounts of
216 heterochromatin, one of them harbouring the NOR. Pair 6 is acrocentric and harbours
217 heterochromatin in its long arm. Finally, the X chromosome is acrocentric and not sub-
218 metacentric.

219 *Herophila fairmairei*. Published data: none. Its karyotype is similar to that of *H. tristis*:
220 $24,XX$ for the females, but the amounts of heterochromatin on the 2 pairs of large acrocentrics
221 are yet more important (Fig. 2.5). Thus, the amplification of heterochromatin on chromosome
222 3 and 4 may be a characteristic of the genus *Herophila*.

223 *Lamia textor*. Published data: none. Its karyotype is composed of 22 chromosomes only, but
224 one pair ($N^{\circ} 8$) is either dicentric or pseudodicentric, so that the number of C-banded
225 “centromeres” is the same as in *Morimus* species (Fig. 2.6). Unfortunately, we could not get

226 males, and female karyotypes did not allow us to identify with certainty the X chromosomes..
227 The size and morphology of autosomes recall those of *Morimus*, but the presence of 4
228 metacentrics suggests that at least 2 inversions separate their karyotypes.

229 ***Dorcadion (Cribridorcadion) thessalicum***. Published data: none. Its karyotype is composed
230 of 22 autosomes, including 2 metacentrics, an acrocentric X and a punctiform Y: 24,XY in the
231 male (Fig. 2.7).

232 ***Dorcadion (Cribridorcadion) equestre***. Published data: none. Its karyotype is composed of
233 24 chromosomes, including 2 pairs of metacentrics, a sub-metacentric X and a punctiform Y:
234 24,XY in the male (Fig. 2.8). The NOR is located on the sub-centromeric position of an
235 acrocentric, probably N°6 or 7.

236 ***Dorcadion (Cribridorcadion) obenbergeri***. Published data: none. Its karyotype is composed
237 of 20 autosomes, a metacentric X and an acrocentric Y (Fig. 2.9). The reduction of the
238 number of autosomes and the enlargement of the sex chromosomes are the consequence of a
239 translocation (fusion) between the X proper and an autosome. Thus, the X is a neo-
240 chromosome. Its short arm is formed by the ancestral X, and its long arm is formed by an
241 ancestral acrocentric, whose size corresponds to N°6 or 7 in other species of *Dorcadion*. The
242 neoY is probably entirely composed by this autosome. This translocation is a synapomorphy.

243 ***Dorcadion (Iberodorcadion) fuliginator***. Published data: none. The number and morphology
244 of its chromosomes are quite similar to that *D. equestre*, except for the X, which is
245 acrocentric: 24,XY. After C-banding, most chromosomes exhibit, in addition to the
246 centromeric bands, one or 2 bands located in intercalary or terminal position of the chromatids
247 (Fig. 1.10). These variable additions of heterochromatin were found in the 3 males studied.

248 ***Neodorcadion bilineatum***. Published data: none. Its karyotype is composed of 22
249 autosomes, with 2 pairs of metacentrics and one pair of sub-metacentric. Chromosome X is
250 sub-metacentric and the Y is punctiform: 24,XY in the males (Fig. 2.11).

251 *Neodorcadion exornatum*. Published data: none. Its karyotype is also composed of 22
252 autosomes with 2 pairs of metacentrics. The X is sub-metacentric and the Y has a
253 heterochromatic short arm: 24,XY. Thus, the karyotypes of the 2 species of *Neodorcadion*
254 differ by at least one peri-centric inversion. In the female studied, there was an additional B
255 chromosome (Fig. 2.12).

256 *Parmena novaki*. Published data: none. Its karyotype is composed of 20 chromosomes, all
257 meta- or submetacentric of progressively decreasing size: 20,XX in the single female studied
258 (Fig. 2.13). The C-banding is limited to the centromeric region.

259 *Monochamus sutor*. Published data: Teppner (1966), Abe et al. (1971), Kudoh et al. (1972),
260 Cesari et al. (2005) and Dutrillaux and Dutrillaux (2014). The karyotype is composed of 18
261 autosomes, all meta- or sub-metacentric. The X chromosome is sub-metacentric and the Y is
262 punctiform: 20,XY in the males (Fig. 2.14). The NOR is sub-centromeric on chromosome 7.
263 There is a size hiatus between chromosomes 1 and 2.

264 *Phytoecia affinis*. Published data: none. Its karyotype is composed of 20 chromosomes, all
265 the autosomes are either metacentric or sub-metacentric of progressively decreasing size:
266 20,XY in the males and 20,XX in the females (Fig. 2.15). The short arm of the X is largely
267 heterochromatic.

268

269 **Nucleotide analysis and phylogenetic reconstruction**

270 Sequences obtained, primers excluded, corresponded to a 525 bp segment. The absence of
271 insertions, deletions or in-frame stop codons within the sequences studied indicated that they
272 correspond to functional mitochondrial *COI* gene fragments and are not derived from nuclear
273 mitochondrial pseudogenes (numts), commonly occur in most eukaryotic species (Richly &
274 Leister, 2004; Cameron *et al.*, 2009). Haplotypes were evaluated and compared to. All
275 haplotypes, after evaluation and comparison to published sequences in Genbank,

276 corresponded to new sequences and thus were submitted to GenBank with accession numbers:
277 MH613717- MH613750.

278 The high percentage (64.7% on average) observed for the A+T content, is a common feature
279 of animal mitochondrial genes (Brown, 1985). Out of the 525 sites, 196 were variable while
280 168 of them were informative for parsimony. Net genetic distances between species ranged
281 from 1.8% (*M. asper* – *M. funereus*) to 26.3% (*Monochamus sutor* – *Dorcadion bilineatum*).
282 Nucleotide divergence within *M. asper* and *M. funereus* were 1.2% and 0.2%, respectively.
283 With some minor differences, the phylogenetic trees based on the Bayesian analysis (tree not
284 shown, available on request), as well as on pairwise haplotype divergence (NJ) (fig. 3),
285 showed similar topologies. The main branches exhibited high bootstrap values (NJ) and high
286 posterior probabilities (Bayesian analysis).

287

288 **Discussion**

289

290 **Chromosome comparisons and evolution.**

291 In Cerambycidae, as in Polyphagan Coleoptera in general, the most frequently reported
292 karyotype formulae are 20,XY and 20,XX, in males and females, respectively, with all or
293 almost all chromosomes metacentric or sub-metacentric (Smith and Virkki, 1978, Dutrillaux
294 and Dutrillaux, 2009). Such karyotypes were observed in most of the tribes of Lamiinae we
295 studied. In our yet unpublished data base, we found it in at least one species of the following
296 tribes: Acanthocini, Acanthoderini, Agapanthini, Desmiphorini, Mesosini, Monochamini,
297 Onciderini, Phytoeciini, Pteropliini, and Saperdini. Thus, it is assumed to represent the
298 ancestral condition, but some variations exist. They consist in an increase of chromosome
299 number to 22, rarely more, generally accompanied by the presence of acrocentrics, which
300 suggests that fissions of the metacentrics occasionally occurred during evolution. In the group

301 of 15 species/sub-species studied here, *P. novaki* and *P. affinis* have conserved a karyotype
302 with 20 chromosomes of progressively decreasing size, probably close to that of the ancestor.
303 With its very large chromosome 1, the karyotype of *M. sutor* is probably derived by a
304 translocation, but it conserved the number of 20 chromosomes. All but 2 of the 12 other
305 species studied here have 24 chromosomes. The 10 karyotypes with 24 chromosomes, which
306 look not much different from each others, may derive from the primitive situation by the
307 fission of 2 metacentrics, which formed 4 acrocentrics. However, their high number of
308 acrocentrics (7 to 9) implies that 3 to 5 peri-centric inversions also occurred. The karyotype
309 with 22 chromosomes of *D. obenbergeri*, obviously apomorphic, derives from that of other
310 *Dorcadion* by a gonosome-autosome translocation. On the whole, the chromosome
311 rearrangements, which originated the resembling karyotypes of genera *Morimus*, *Herophila*
312 and *Dorcadion*, very probably occurred in common ancestors. Thus, these genera form a
313 monophyletic group. The chromosomal relationships between *Lamia textor* and others are less
314 obvious. The karyotype of this species, with 22 chromosomes, looks intermediary between
315 those of the groups with 20 and 24 chromosomes, but one pair looks dicentric, or pseudo-
316 dicentric, as defined in ISCN (1985), with 2 primary constrictions and 2 C-bands (Fig. 2.6).
317 Thus, it has 24 potential centromeres, and may derive by a termino-terminal fusion of 2
318 acrocentric chromosomes from a karyotype with 24 chromosomes. Because of the frequent
319 involvement of the X chromosome in translocations in Coleoptera (Dutrillaux and Dutrillaux,
320 2009), as here in *D. obenbergeri*, it is possible that the proper X forms one arm of this
321 pseudo-dicentric.

322 In conclusion, the lack of available probes makes impossible to perform a precise
323 chromosome identification by in situ hybridization, and thus, to reconstruct a phylogeny by
324 chromosome comparison. Nevertheless, the great resemblance of their karyotypes are strong
325 arguments in favor of a monophyletic origin of genera *Morimus*, *Herophila*, *Dorcadion* and

326 *Lamia*, which share similar derived features compared to other Lamiinae. At difference, the
327 karyotypes composed of 20 chromosomes, here observed in *P.novaki*, *M. sutor* and *P. affinis*
328 share many features, with many other Cerambycids. We conclude that they underwent few
329 gross chromosome changes during evolution.

330

331 **CO1 sequence comparisons**

332 In a recent study Solano et al. (2013) analysed 65 specimens of *M. funereus* sampled in Italy,
333 Balkans and Turkey by applying COI and ITS2 molecular markers. Unfortunately, although
334 they found 39 haplotypes for COI, the overlap between this set of sequences and ours was
335 practically nonexistent (of approximately 10 nucleotides), making it impossible to compare
336 between them. However, a striking difference between the two studies was the 10-fold higher
337 nucleotide diversity assessed for *M. funereus* ($\pi= 0.0226$) in comparison to our data ($\pi=$
338 0.002). A possible explanation for this discrepancy could be the much larger range of
339 sampling in the study of Solano et al. (2013).

340 Sequencing does not contradict chromosome data, but it provides a better resolution. Species
341 belonging to the various tribes of Lamiinae are well separated, and there is a common trunk
342 for genera *Morimus*, *Lamia*, *Herophila* and *Dorcadion*. The splitting of *Dorcadion* in
343 *Iberodorcadion*, *Neodorcadion* and other *Dorcadion* will be discussed elsewhere, with the
344 study of many more species. The 2 species of *Herophila* are monophyletic, and also well
345 individualised. CO1 sequences of specimens of *H. fairmairei*, a rare and very localised
346 species, appear to be fairly close to each other, while those of *H. tristis*, a largely spread
347 species, are more diverse. Thus, both bio-geographic and genetic data suggest that *H.*
348 *fairmairei* may be a localised vicariant of *H. tristis*.

349 The genus *Morimus* is fairly homogeneous, although separated into two groups, with *M. asper*
350 on one side and *M. funereus* and *M. ganglbaueri* on the other side. CO1 sequences did not

351 accumulate many mutations separating French and Greek specimens of *M. asper*. The
352 position of the French specimens of *M. asper* in the cladogram is among the most derived
353 one. This is compatible with a radiation of the genus *Morimus* from Balkans, or more oriental
354 countries, to western countries such as France and Spain, which would be the extremity of the
355 radiation and the most recently colonised countries. The similarity of haplotypes found in *M.*
356 *ganglbaueri* and *M. funereus* is interesting. *M. ganglbaueri* shares rather more morphological
357 characters with *M. asper* (long antennas and granulation of elytra), than with *M. funereus*
358 (density and colour of pilosity). By these mixed characters and its geographic repartition,
359 surrounded by that of *M. funereus* on the East and *M. asper*, on the South and West, *M.*
360 *ganglbaueri* might have a hybrid origin. In this hypothesis, the similarity of mitochondrial
361 CO1 sequence with *M. funereus* could indicate that this species was originally the maternal
362 contributor. Our breeding experiments show that hetero-specific couples can occur, but we
363 could not formally demonstrate their fertility.

364 As suggested by chromosome data, the CO1 sequence places *Lamia textor* within the group
365 *Morimus*, *Herophila* and *Dorcadion*, far from other taxa. More precisely, *L. textor* looks very
366 close to *Dorcadion*.

367

368

369 **Comparison between CO1 and chromosomal data**

370 There is no major discrepancy between the two sets of data, but the mutations of the CO1
371 sequence are much more discriminating than the alterations of chromosomes for establishing
372 a phylogeny. Thus, it was possible use the cladogram obtained with CO1 analysis for
373 indicating the minimum number of chromosome rearrangements necessary to pass from one
374 karyotype to another (Fig. 3). The maximum number of chromosome changes should have
375 occurred in the common trunk for *Morimus*, *Herophila*, *Lamia* and *Dorcadion*, which fits

376 with CO1 data. It persists however 2 major questionings: 1) *D.I. fugilinator* has the same
377 karyotype than the other species of *Dorcadion*, if we omit heterochromatin variations,
378 although CO1 data place it near *Herophila*. The explanation may be that the karyotype of
379 *Dorcadion* is also that of the ancestors of both *Herophilus* and *Morimus*, but the study of
380 more species of *Iberodorcation* is necessary for more supported conclusions. 2) CO1 data
381 place *L. textor* near *Dorcadion* whereas its karyotype seems to have conserved more ancestral
382 features. The 2 inversions indicated after bifurcation (Fig. 3) could be reverse, but
383 chromosome interpretation would be simpler if *L. textor* was branched between bifurcations c
384 and d before the acquisition of 2 of the 4 inversions. The study of more specimens, males in
385 particular, is urgently needed. By evaluating the convergence of chromosomal and molecular
386 data, we believe that, despite its limitations when applied solely (e.g. selective sweeps,
387 inability to identify hybridization), the CO1 marker provides the required signal to reconstruct
388 the phylogenetic relationships between the genera under study. In the last decade, there is
389 growing evidence about the importance of the CO1 gene and its unique barcoding attributes
390 are believed to have arisen from its implication to the process of speciation (Gershoni et al,
391 2009). The species-specific mitonuclear coadaptations that occur to maintain the functionality
392 of the Oxidative Phosphorylation are thought to be a major force of speciation (Lane, 2009).
393 The CO1 gene possesses a “special place” in the above mentioned since it encodes for a
394 catalytic subunit of cytochrome c oxidase, which is a key OXPHOS regulator (Hill, 2016). As
395 he proposes: “...it is no coincidence that the rate-controlling gene in the ECT that can enable
396 respiratory adaptation to novel environments is also an effective DNA barcode for animals.”

397

398 **Conclusion**

399 The comparisons of CO1 mitochondrial gene sequences and karyotypes give convergent data,
400 which indicate an undisputable monophyletic origin of genera *Morimus*, *Herophila*, *Lamia*

401 and *Dorcadion*, at a large phylogenetic distance from other genera such as *Monochamus*.
 402 This does not support classifications, which put together *Lamia* and *Monochamus* in a same
 403 tribe, whatever its name Monochamini, Lamiini or Agniini. The nomenclature problem seems
 404 to be historical: *Lamia*, considered as the genus type of Lamiinae, logically should remain in
 405 the *Lamiini* tribe. It should better have been grouped with *Dorcadion* (Dorcadionini) and
 406 *Morimus/Herophila* (Phryssomini), but instead, it has been always grouped with many other
 407 taxa, such as *Monochamus*, which have very loose phylogenetic relationships. Thus, two
 408 options can be proposed: either *Lamia* with only a few closely related genera remain in a
 409 restricted *Lamiini* tribe, or the tribes *Phryssomini* and *Dorcadionini* are suppressed and their
 410 species join these few *lamiini* to form a monophyletic new *Lamiini* tribe. This would provide
 411 a logical basis for further classifications

412

413 Figures captions

414

415 **Figure 1**

416 Map of Greece with indications by letters of the places of sampling. The letters correspond to
 417 those indicated in the cladogram of figure 3: a-f: *M. asper*; g-k: *M. funereus*; l: *M.*
 418 *ganglbaueri*; m: *D.I. fuliginator*; n,o: *H.tristis*; p: *H. fairmairei*; q: *Neodorcadion bilineatum*;
 419 r: *Neodorcadion exornatum*; s: *L. textor*; t: *D. C. thessalicum*; u: *D. C. equestre*; v: *D.C.*
 420 *obenbergeri*; w: *P. affinis*; x: *P. novaki*; Y: *M. sutor*.

421 **Figure 2**

422 Karyotypes after Giemsa staining (13) or C-banding (all others). 1: *M. asper*; 2: *M.*
 423 *ganglbaueri*; 3: *M. funereus*; 4: *H. tristis*; 5: *H. fairmairei*; 6: *L. textor*; 7: *D. C. thessalicum*;
 424 8: *D. C. equestre*; 9: *D. C. obenbergeri*; 10: *D. I. fuliginator*; 11: *N. bilineatum*; 12: *N.*
 425 *exornatum*; 13: *P. novaki*; 14: *M. sutor*; 15: *P. affinis*.

426 **Figure 3**

427 Phylogenetic tree constructed with the NJ algorithm. Small letters on the left correspond to
428 specimens collected at the locations indicated at Figure 1. Chromosome rearrangements
429 separating the karyotypes are indicated but were not considered to establish the tree. F:fission;
430 I: pericentric inversion, T: translocation; D: Dicentric or pseudodicentric; H: large
431 heterochromatin addition.

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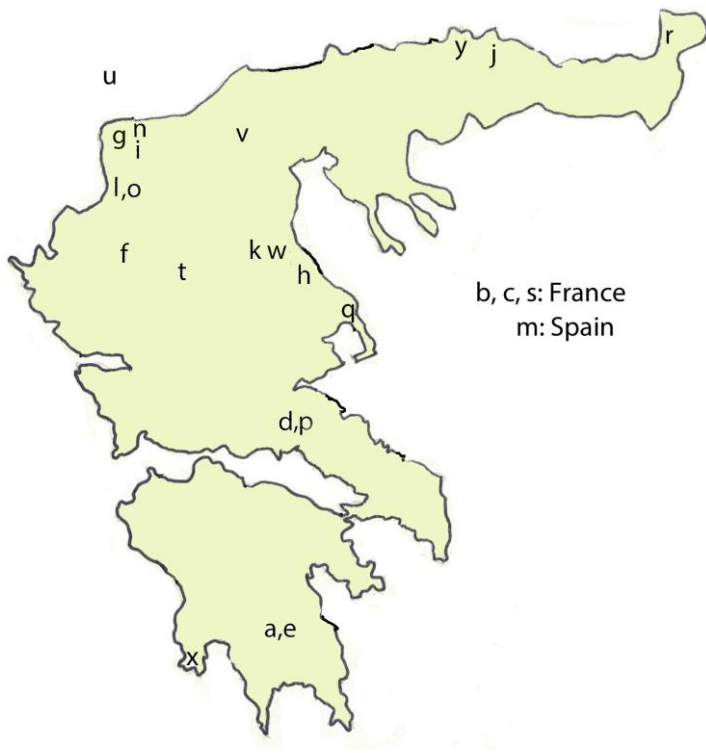
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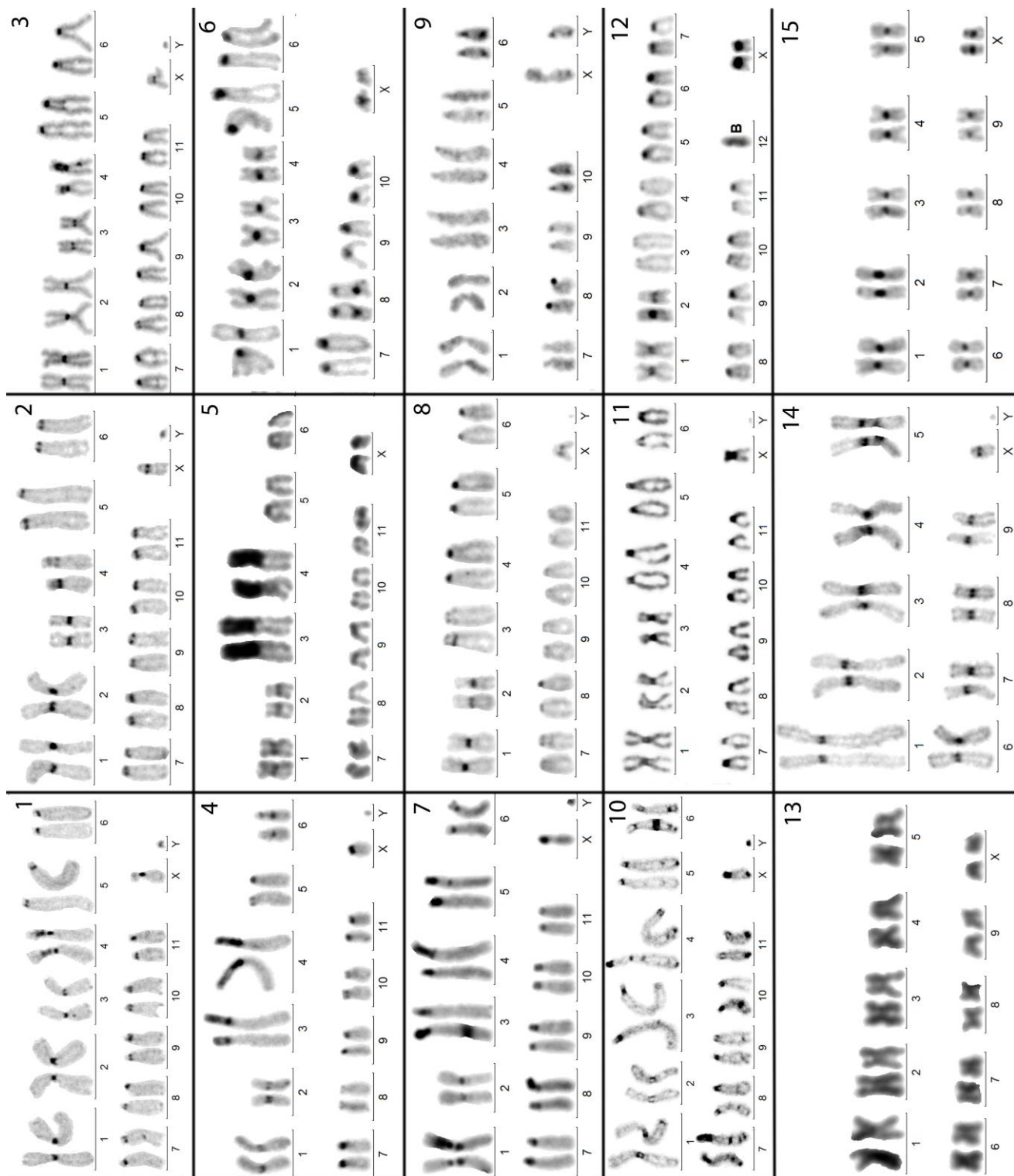
487 **Figure 1**



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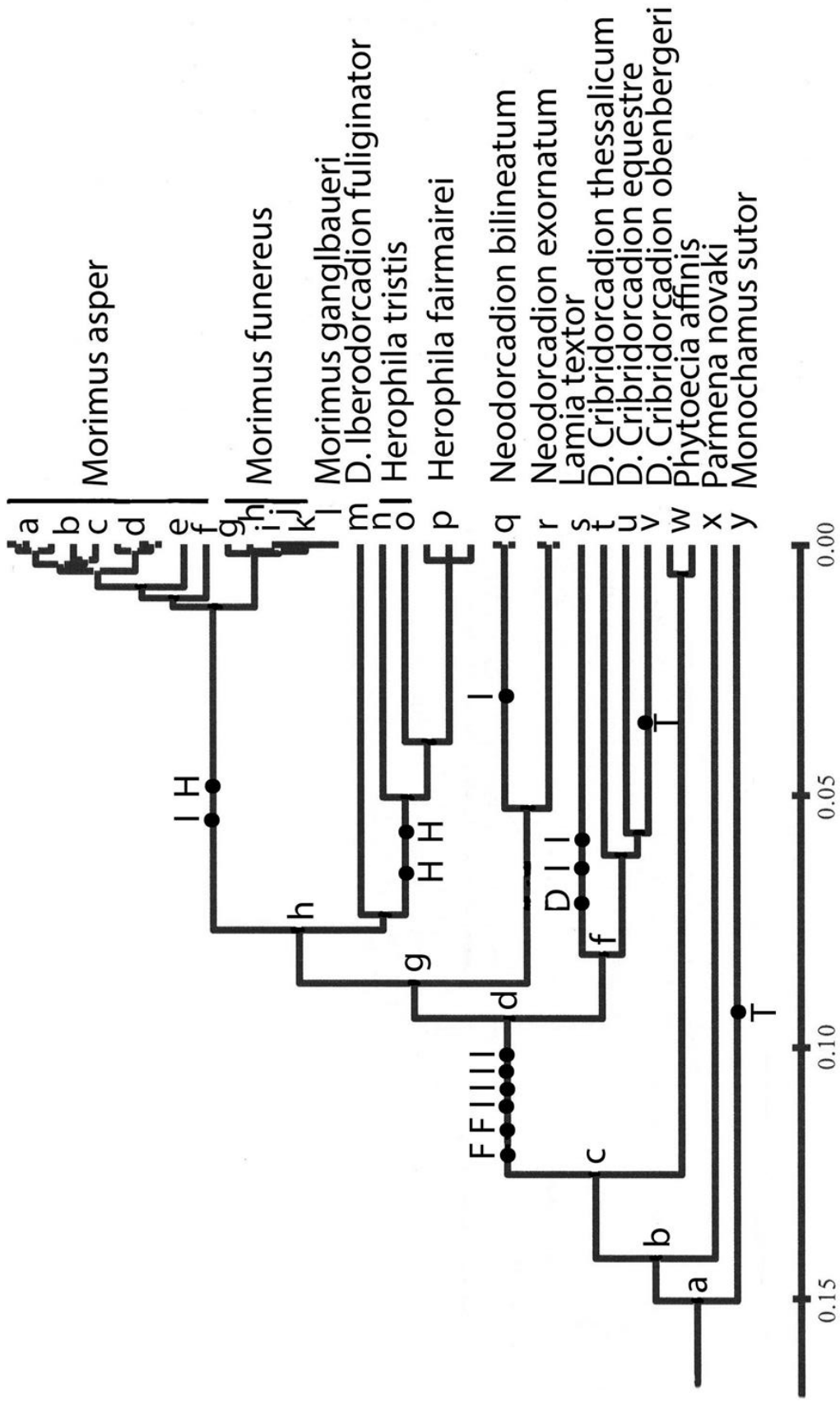
490 **Figure 2**



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492

493 Figure 3



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