1	Time-scaled phylogenetic analysis of extant Lamiinae (Coleoptera, Cerambycidae)
2	species of East of Marmara Basin, Türkiye, and their evolutionary similarity with
3	the Eurasian congeners
4	Havva Kübra SOYDABAŞ-AYOUB*, Fevzi UÇKAN,
5	Department of Biology, Faculty of Arts and Science, Kocaeli University, Kocaeli,
6	Türkiye
7	*Correspondence: havvasoydabas@gmail.com
8	Abstract: The subfamily Lamiinae (Cerambycidae, Coleoptera) is striking due to its
9	morphological diversity and species richness with intricate phylogenetic relationships.
10	We inferred the phylogeny and evolutionary history of extant species of East of Marmara
11	Basin, Türkiye, from the tribes Acanthocinini, Acanthoderini, Agapanthiini, Batocerini,
12	Dorcadionini, Lamiini, Mesosini, Monochamini, Phytoeciini, Phrynetini, Pogonocherini
13	(including Exocentrini) and Saperdini using partial mitochondrial cytochrome c oxidase-I
14	(COI) and 16S rRNA and nuclear 28S rRNA gene regions (2257 base pair alignment
15	length). The most recent common ancestor (MRCA) of Lamiinae members included in
16	the analyses was dated ~127 million years ago (Mya) in the Cretaceous. The MRCA of
17	Dorcadionini, Lamiini and Monochamini was younger than the common ancestors of the
18	other close tribes. There was a concurrence between resolutions of Maximum Likelihood
19	(ML) and Bayesian analyses on the affiliations of Dorcadionini and Monochamini to
20	Lamiini and the proximity of Batocerini to Lamiini, Acanthocinini to Acanthoderini,
21	Phrynetini to Pogonocherini, and Phytoeciini to Saperdini. The COI-based Neighbor-
22	Joining and ML gene trees suggest that the closest relatives of the extant Lamiinae
23	species of East of Marmara Basin were the European conspecifics or congeners.
24	Moreover, Paraleprodera and Lamia (Lamiini) were sisters to Imantocera (Gnomini),

1	Oberea (Obereini) to Phytoecia	Phytoeciini), and Hippe	opsis (Agapanthiini) to
2	Omosarotes	singularis	Pascoe,

1 1860 (Acanthomerosternoplini). Our results support Dorcadionini, Gnomini and 2 Monochamini as synonyms of Lamiini; and Obereini and Phytoeciini of Saperdini and 3 suggest that the emergence of the living tribes included in this study was during 4 Paleogene, and their intrageneric diversifications occurred during Cenozoic, mostly 5 Neogene.

Keywords: Cretaceous, Molecular clock, Paleogene, intrageneric diversification times,
Neogene

8 1. Introduction

Cerambycidae, also known as long-horned wood-boring beetles, is the species-rich 9 10 family of the superfamily Chrysomeloidea (Coleoptera, Phytophaga), with about 34,000 described species; among them, around 20,000 extant species belong to subfamily 11 12 Lamiinae (Tavakilian and Chevillotte, 2022). The family comprises mainly herbivorous beetles distributed in all biogeographic realms except Antarctica (Monné et al., 2017). 13 Some species are considered pests of wood due to their larval feeding habits; however, 14 15 beyond this, they have pivotal roles in sustaining the harmony of the forest ecosystem, 16 such as pollination and decaying wood (Linsley, 1959).

17 The prominent dependence of cerambycids on the plant has led to investigations of their 18 evolution as a part of the herbivorous beetles (Phytophaga) clade, whose over-19 diversification has been mainly ascribed to their adaptive radiation (Farrell, 1998; 20 McKenna et al.; 2015; Zhang et al., 2018; McKenna et al. 2019). According to Farrell 21 (1998), basal lineages of cerambycids emerged in the Jurassic, and the ancestral lineages 22 were associated with gymnosperms. The enhanced net diversification rate of primitively 23 angiosperm-associated taxa is related to a series of adaptive radiations of beetles onto 24 angiosperm.

1 In contrast to him, Gómez-Zurita et al. (2007) suggested that the origin of the Chrysomelidae is 73–79 Mya and the origin of cerambycids is Late Cretaceous about 80 2 Mya, thus cannot be associated with diversification of Early Cretaceous angiosperm 3 4 lineage. Hunt et al. (2017) placed the origin of Coleoptera at 285 Mya, dated the origin of 5 cerambycids at 203 Mya, and dated the first appearance of most of the modern lineages 6 of beetles in the Jurassic. Despite similar dating with Farrell (1998), Hunt et al. (2017) 7 concluded that the underlying reason for the success of beetles is neither the net 8 diversification rate nor the Cretaceous rise of angiosperms, instead sustained 9 diversification in various niches. Then the divergence time of Chrysomeloidea was recalibrated by Wang et al. (2014) due to a discovery of the known earliest cerambycid 10 11 fossil, Cretoprionus liutiaogouensis Wang, et al., 2013 (Prioninae) from the Early Cretaceous of China. According to their estimation, the origin of modern Cerambycidae 12 was the Late Triassic (~210 Mya), older than the estimates of Gómez-Zurita et al. (2007), 13 and all subfamilies arose by the mid-Cretaceous. Then, again, the estimation of the 14 15 emergence time of the family was shifted to the beginning of the Early Cretaceous around 16 145 Mya by Zhang et al. (2018). McKenna et al. (2019) dated the origin of Coleoptera to 17 the Carboniferous about 327 Mya. They also suggested that the diversification rate within Polyphaga increased in the Late Carboniferous by about 305 Mya. The origins of most of 18 the extant beetle families, including Cerambycidae, appeared before the end of the 19 20 Cretaceous, and the emergence of cerambycids was around 160 Mya (McKenna et al., 21 2019). Nie et al. (2021) dated the emergence of the ancestor of the crown group of the 22 subfamily Lamiinae at 132.0 Mya.

Besides evolutionary relationships, the studies inferring phylogenetic relationships of the
subfamilies of Cerambycidae are primarily focused on the higher taxa. The studies rely

1 on either morphological or molecular synapomorphic characters or both (Napp, 1994; 2 Švácha and Lawrence, 2014; Wei et al., 2014; Haddad et al., 2018; Nie et al., 2021), have 3 reached almost complete unanimity on the monophyly of the subfamily Lamiinae. 4 However, the morphological and molecular studies conflict in who is the sister clade of 5 this subfamily. In contrast to the previous morphological studies (Villiers, 1978; Napp, 6 1994) that suggest Cerambycinae as the sister of Lamiinae, molecular phylogenetic 7 analysis (Gómez-Zurita et al., 2007; Marvaldi et al., 2009; Haddad et al., 2018; Nie et al., 8 2021) have concurrence that Spondylidinae is the sister clade of Lamiinae.

9 Recently, some studies took initial steps to resolve the tribal-level relationships of 10 Lamiinae based on genetic data. de Santana Souza et al. (2020) inferred using 11 mitochondrial and nuclear marker DNA sequences that among the phylogenetically analyzed 46 tribes, Astathini, Batocerini, Ceroplesini, Colobotheini, Compsosomatini, 12 Dorcadionini, Lamiini, Mesosini, Obereini, and Polyrhaphidini are monophyletic; but, 13 some of widely distributed or diverse tribes such as Acanthocinini, Acanthoderini, 14 15 Agapanthiini, Apomecynini, Desmiphorini, Dorcaschematini, Enicodini, Hemilophini, 16 Monochamini, Onciderini, Parmenini, Phytoeciini, Pogonocherini, Pteropliini, and 17 Saperdini are polyphyletic. Ren et al. (2021) presented the sisterships of Ceroplesini and Agapanthiini, Mesosini and Pteropliini, Saperdini and Phytoeciini, Batocerini and 18 19 Lamiini, and Apomecynini and Acanthocinini based on analyzed 13 protein-coding genes of mitogenomes of 12 tribes' representatives. Ashman et al. (2022) reported that 20 21 Acanthocinini, Apomecynini, Parmenini, Desmiphorini, Lamiini and Pteropliini are 22 polyphyletic, relying on the phylogenetic analysis of hundreds of nuclear gene regions, 23 including representatives of 14 tribes, mostly Australian. The present study aims to figure out the evolutionary closeness of the Lamiinae species that presently occur in the East of 24

1 Marmara Basin, Türkiye, with their Eurasian relatives using partial mitochondrial 2 cytochrome c oxidase-I (COI) gene region and to contribute to phylogenetic relationships 3 and the evolutionary history of tribes Acanthocinini, Acanthoderini, Agapanthiini, 4 Monochamini, Phytoeciini, Pogonocherini, and Saperdini using partial mitochondrial 5 COI and 16S rRNA and nuclear 28S rRNA gene regions.

2. 6

Materials and Methods

7 Specimens were collected from timber yards, wood processing plants, suburban areas and 8 forests between 2016 and 2019 in East of Marmara Basin, Türkiye (Supplementary Table 9 S1), a remarkable region for insect biodiversity due to its geographic position and climatic (Cakmak et al., 2019); vegetative (Atak et al., 2021), and mercantile (Soydabas-10 11 Ayoub et al., 2022) characteristics. Ethanol and α -pinene tubes were used within three funnel traps. Insect nets were used for flower-visiting species. Phryneta leprosa (Castilla-12 borer) was intercepted in Derince port on timber imported from Cameroon. Batocera 13 rufomaculata (tropical fig-borer) was caught coincidentally in Diyarbakır, Türkiye. 14 15 Morphological identifications were carried out with the guidance of Breuning (1951), 16 Bílý and Mehl (1989), Bense (1995), Sama (2002), Wallin, Nylander, and Kvamme 17 (2009), and Özdikmen (2013) under a stereomicroscope (Olympus SZ51, Japan) enhanced by Olympus 110AL2X WD38 auxiliary macro lens. Specimens were stored in 18 99% ethanol at -20 °C pending DNA extraction. 19

Depending on the specimen sizes, muscle tissues of coxa or antennae were shredded on 20 21 disposable sterile slides by disposable scalpels. Then transferred into a microcentrifuge 22 tube including the lysis buffer (8 mM dithiothreitol (DTT), 2% sodium dodecyl sulphate 23 (SDS), 100 mM NaCl, 3 mM CaCl2, dissolved in 100 mM Tris buffer (pH 8) and 200 µg/mL proteinase K) (Soydabas-Ayoub, 2021) and kept in Eppendorf, Germany, 24

Thermomixer 5350 heater at 37°C until whole tissue whittle down. Genomic DNA was
 extracted by following the phenol:chloroform:isoamyl alcohol (25:24:1) protocol
 (Sambrook and Russell, 2006).

4 Polymerase chain reaction (PCR) products were amplified in a total volume of 25µL with 5 QuickLoad® Taq 2X Master Mix (New England Biolabs Inc., USA, catalogue number 6 M0271L) and 0.2 μ M primer for each pair, with the addition of 0.06 mg/mL bovine 7 serum albumin. The primer pairs HCO2198-LCO1490 (Folmer et al., 1994) for the 8 mitochondrial COI gene region, LR-J-12887- LR-N-13398 (Yoon et al., 2001) for mitochondrial 16S rRNA gene region, and LSU D1, D2 fw1: 56-74 and LSU D1, D2r 9 rev2: 1048-1067 (Sonnenberg et al., 2007) for nuclear 28S rRNA gene regions were used 10 in amplification. Mitochondrial COI and 16S rRNA gene regions were amplified by using 11 the same thermocycling parameters as 1 min at 95 °C, five cycles of 30 s at 95 °C, 1 min 12 at 46 °C, 1 min at 72 °C, 30 cycles of 30 s at 95 °C, 1 min at 51°C, 1 min at 72 and 10 13 min at 72 °C (Cakmak et al., 2020). The thermocycling parameters used for amplification 14 15 of the nuclear 28S rRNA gene region were 1 min at 95 °C, four cycles of 30 s at 95 °C, 1 16 min at 57,3 °C, 1 min at 72 °C, and 20 cycles of 30 s at 94 °C, 1 min at 61,7 °C, 1 min at 17 72 °C and 10 min at 72 °C (Soydabaş-Ayoub et al., 2022). ExoSAP-ITTM Clean-Up Reagent was used for purification according to manufacturer instructions. Sequencing 18 19 was performed for both directions by ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) at Macrogen Laboratory, Holland. 20

Geneious Prime v2019.2.1 (Kearse et al., 2012) was used for processing raw data. Prior to BLAST searches of the contigs of high-quality (HQ% >90) chromatograms, the primer regions and the leftover extensions following primer sequences were trimmed.

Sambrook J, Russell DW (2006). Purification of nucleic acids by extraction with phenol:chloroform.ColdSpringHarborProtocols,2006[online]www.cshprotocols.cshlp.org/content/2006/1/pdb.prot4455[accessed 12 March 2019]6

1 The heterozygote bases in nuclear gene sequences were named based on the IUPAC 2 (International Union of Pure and Applied Chemistry) base nomenclature code system. A 3 total of 143 obtained sequences were deposited in GenBank under the accession numbers 4 OP279135-OP279183, OP279535-OP279581 and OP279486-OP279532 for 5 mitochondrial COI, 16S rRNA and nuclear 28S rRNA gene regions, respectively 6 (Supplementary Table S1). Two datasets were prepared for the phylogenetic analysis. 7 The first one was a global mitochondrial COI sequence dataset of the subfamily 8 Lamiinae, which was configured by combining the sequences obtained in this study and retrieved from the BOLD taxonomy archive. To improve the dataset's reliability, 9 10 sequences shorter than 658 bp were discarded. A representative was selected for each available species from each zoogeographic region based on Löbl and Smetana (2010) 11 12 (i.e., Neotropical, Nearctic, Afrotropical, Oriental Australian, Palearctic: Europe, Asia, North Africa) (Supplementary Table S2). 13

The second dataset consisted of two mitochondrial and one nuclear marker sequences; all were produced in this study (Supplementary Table S1). The mitochondrial *COI*, *16S rRNA*, and nuclear *28S rRNA* gene regions were concatenated in Geneious Prime v2019.2.1 (Kearse et al., 2012) and aligned by MUSCLE v3.8.425 (Edgar, 2004). Model selections were carried out by PartitionFinder 2 (Lanfear et al., 2017), the basis of AICc, by greedy search.

20 Phylogenetic trees were obtained using Maximum Likelihood (ML) and Neighbor-21 Joining (NJ) approaches. ML analysis was conducted by using the PhyML (Guindon et 22 al., 2010) plug-in of Geneious Prime v2021 for spliced dataset (Supplementary Table S1) 23 of three gene regions (2257 bp) with 34 terminal, produced in this study; and a global 24 dataset of the mitochondrial *COI* gene region, including the sequences produced in this

1 study (Supplementary Table S1) and retrieved from databases (658 bp) with a total of 441 2 terminal (Supplementary Table S2). For both datasets, ML analyses were carried out by 3 10,000 bootstrap pseudo-replicates with estimated parameters and the Tamura-Nei 4 (TN93) substitution model for unpartitioned data. The outgroups used in phylogenetic 5 analyses were selected from the closest relatives according to Haddad et al. (2018): Spondylis buprestoides (Linnaeus, 1758) (Spondylidinae: Spondylidini), Arhopalus 6 rusticus (Linnaeus, 1758) (Spondylidinae: Asemini). NJ analysis was conducted by tree 7 8 builder in Geneious Prime v2021 with 10,000 bootstrap pseudo-replicates and TN93 substitution model for only the global COI dataset (Supplementary Table S1). 9

The time-scaled Bayesian analysis was conducted by BEAST v1.10.4 (Drummond and 10 11 Rambaut, 2007) under the Yule speciation process (Gernhard, 2008) and uncorrelated relaxed clock model with lognormal distribution (Drummond et al., 2006). Molecular 12 clock estimation was calibrated considering fossil records and chronograms of recent 13 studies (McKenna et al., 2019; Nie et al., 2021; Ashman et al., 2022). The normal 14 15 distribution with $\pm 5\%$ standard deviation was used for calibrating the root ages. The 16 mean ages of the most common recent ancestor (MRCA) were set to 147.03, 138.1, and 17 132.0 Mya for the lamiine clade (Lamiinae+Spondylidinae), Spondylidinae and Laminae, respectively based on Nie et al. (2021). The root age (crown lamiine clade) was 18 constrained to 180 Mya (McKenna et al., 2019). The lower bounds of MRCAs of 19 Spondylidinae and Laminae were truncated to 34 Mya and 57 Mya, respectively, relying 20 21 on fossil records of Arhopalus pavitus Cockerell 1927 from Eocene of Colorado 22 (Cockerell, 1927) and Palaeoncoderes and Prolamioides from Paleocene of France (Piton 23 and Théobald, 1937).

1 The Jukes-Cantor (JC) substitution model was selected after trial runs to determine the 2 best-fitting model since it provided higher posterior probabilities than other substitution 3 models available in BEAST v1.10.4 (HKY, GTR, and TN93). Auto-optimized classical 4 operator mix parameters were used for two Bayesian analyses, run for 100 million 5 MCMC generations, and sampled every 1000 steps; of these, 10% were discarded as 6 burn-in. LogCombiner v1.10.4 merged the outputs, and the maximum clade credibility 7 (MCC) trees resulting from each run were interpreted by TreeAnnotator v1.10.4 in the 8 BEAST package. Tracer 1.7 (Rambaut et al., 2018) was used to assess if the implemented 9 parameters provide effective sample sizes (ESSs). The MCC trees were annotated in FigTree v1.4.4 (Rambaut, 2014), and the visual adjustments were performed in 10 11 CorelDraw v.1512.

12 **3. Results**

13 **3.1.** Phylogenetic Analysis

The hypothetical phylograms resulting from the NJ and ML analyses of the global dataset 14 15 of COI barcode region adumbrated relationships, particularly at the generic level. All 16 specimens obtained in this study from the East of Marmara Basin were clustered with the 17 European conspecifics, congeners or relatives (Supplementary Figure S1, Supplementary Figure S2). The phylogram hypothesized by the ML approach of spliced datasets of 18 19 mitochondrial COI and 16S rRNA and nuclear 28S rRNA gene regions (Supplementary Table S1) of Lamiinae schemed a pattern as follows: The first main branch including 20 21 [(Lamiini+Dorcadionini) + (Monochamiini+Batocerini)] + Mesosini, the second 22 Phrynetini+Pogonocherini (including Exocentrini), the third Agapanthiini, fourth 23 Acanthocinini+Acanthoderini, and the last one Saperdini+Phytoeciini (Figure 1). For traceability, we mostly used generic names of the representatives of tribes in thefollowing text.

3 The COI-based NJ and ML gene trees supported the affiliation of the genera Morimus 4 (Lamiini) and Dorcadion (Dorcadionini), besides the genus Iberodorcadion 5 (Dorcadionini) nested in the same group, sister to *Morimus+Dorcadion* branch. The other genera splinted from the same node in the NJ tree were Paraleprodera and Lamia 6 7 (Lamiini), Imantocera (Gnomini), Batocera and Apriona (Batocerini) (Supplementary 8 Figure S1 and Supplementary Figure S2). Peblephaeus (Lamiini) was stated at the basalmost of the group at the NJ tree (Supplementary Figure S1). Mesosa obscuricornis was 9 10 clustered with congeners and Synaphaeta (Mesosini) and Acalolepta (Lamiini) 11 (Supplementary Figure S1 and Supplementary Figure S2).

The Phrynetini+Pogonocherini branch was recovered as the sister group of Lamiini and the relatives mentioned above (Figure 1). The genus *Exocentrus*, which is a member of the tribe Pogonocheriini (or Exocentrini), was nested within this cluster with a 70% bootstrap support at the ML tree of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (Figure 1).

17 At the COI-based NJ and ML trees, the members of the genus Pogonocherus were clustered all together, split away from other members of Pogonocherini. This cluster was 18 19 the basal-most of the COI-based ML tree. The two genera in Pogonocherini, Ecyrus and 20 *Exocentrus*, were clustered with *Rosalba sp.* (Apomecynini) and *Bactriola* sp. 21 (Forsteriini) at the COI-based ML tree Supplementary Figure S2). The genus Phryneta 22 was clustered with the members of the genera Anaesthetis and Pseudanaesthetis from the 23 tribe Desmiphorini (Supplementary Figure S2), while *Exocentrus* was clustered with Desmiphora (Desmiphorini) at the COI-based NJ tree (Supplementary Figure S1). 24

1 The tribe Agapanthini was represented by the genus Agapantia at the ML tree of the 2 spliced datasets of mitochondrial COI and 16S rRNA and nuclear 28S rRNA gene regions, 3 stated at the base of the groups mentioned above with trivial statistical support (Figure 1). 4 At the COI-based NJ and COI-based ML trees, two other species from Agapanthiini, 5 Calamobius filum (Rossi, 1790) and Pothyne virginalis Takakuwa and Kusama, 1979 6 were clustered in the Agapantia genus group (Supplementary Figure S1, Supplementary 7 Figure S2). 8 The sole representative of the tribe Acanthoderini Aegomorphus clavipes, stated at the 9 base of the Acanthocinini and Acanthoderini group at the ML tree of spliced datasets of mitochondrial COI and 16S rRNA and nuclear 28S rRNA gene regions (Figure 1). At the 10 11 COI-based NJ and COI-based ML trees Aegomorphus modestus (Blais, 1817) was joined 12 to A. clavipes. They were clustered with other Acanthoderini members, such as genera Acanthoderes, Paradiscopus, Psapharochrus and Steirastoma (Supplementary Figure S1, 13 Supplementary Figure S2). 14 15 The basal-most branch of the ML tree of the spliced datasets of mitochondrial COI and 16 16S rRNA and nuclear 28S rRNA gene regions included Saperdini and Phytoeciini 17 (Figure 1). At the tree resulting from COI-based NJ and COI-based ML analysis, the genus Saperda group included the members of genera Eutetrapha, Glenea, Thyestilla and 18 19 Stenostola from the tribe Saperdini. In the genus group of *Phytoecia*, the genus Oberea 20 (Obereini) was nested. These two groups were stated as sister clades (Supplementary 21 Figure S1, Supplementary Figure S2).

22 **3.2.** Divergence Time Estimation

The time-scaled Bayesian analysis of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (Supplementary Table S1) suggested a mostly

1 congruent topology with the ML analysis (Figure 1, Figure 2). The MRCA of Lamiinae 2 rely on the taxa involved in the analysis, raised around the Early Cretaceous, 127.49 Mya 3 (95% highest posterior density (HPD) interval 117.95-137.57 Mya). The common ancestor 4 of the Lamiini and its close relatives (Dorcadionini, Monochamiini, Batocerini and 5 Mesosini appeared 66.77 Mya (95% HPD: 42.34-92.92 Mya) around the Late Cretaceous 6 to Eocene epoch of the Paleogene. Also, appearing times of the earliest common 7 ancestors of the other groups were from Late Cretaceous to the different epochs of the 8 Paleogene. Phrynetini+Pogonocherini (including Exocentrini) and 9 Acanthocinini+Acanthoderini groups emerged in Late Cretaceous to Paleogene 58.85 10 Mya (HPD: 29.25-87.85 Mya) and 77.71 Mya (95% HPD: 40.68-111.73 Mya), respectively. The MRCA of Saperdini+Phytoeciini emerged in 65.24 Mya (95% HPD: 11 12 23.06-111.61 Mya), somewhere from the mid-Cretaceous to the end of Oligocene. While the emerging time of the MRCA of the Agapanthia genus group was 54.74 Mya (95%) 13 HPD: 27.5-87.9 Mya), the emerging time of the MRCA of the tribes Dorcadionini, 14 15 Lamiini, and Monochamini was 41.28 Mya (95% HPD: 21.51-63.81 Mya). Besides, the 16 MRCA of these three tribes was younger than all MRCAs of the other tribes (Figure 2). 17 The intrageneric diversification relies on the taxa involved in the analysis that occurred during Cenozoic. The members of Agapanthia arose during Paleogene 54.74 Mya (95% 18 19 HPD: 27.5-87.9 Mya), earlier than Phytoecia members 23.13 Mya (95% HPD: 3.91-20 54.16 Mya.) The last species emerging within our sampling corresponded to the Neogene 21 and occurred within the genus Dorcadion 14.34 Mya (95% HPD: 4.04-29.83 Mya).

22 **4. Discussion**

The previous studies contributing knowledge of the evolutionary history and
phylogenetic relationships of Lamiinae are mainly dealing with higher-level relationships

1 and covering only a few laminin taxa (Farrell, 1998; Gómez-Zurita et al., 2007; Hunt et 2 al., 2007; Wang et al., 2014; Haddad et al., 2018; Zhang et al., 2018). Since the inception 3 of diversification of herbivorous beetles has been set on relatively solid ground, the 4 studies are bending to lower taxonomic levels. Phylogeny of Lamiinae, especially at 5 tribal and generic levels, has remarkable progress owing to de Santana Souza et al. 6 (2020), Ashman et al. (2022) and Dascălu et al. (2022). However, the relationships at 7 tribal and generic levels still require intense effort due to the species richness, remarkable 8 diversity and the worldwide distribution of the subfamily. The present study contributes to understanding tribal and generic level phylogenetic relationships and the evolutionary 9 10 history of Lamiinae using Eurasian species as the sampling allows.

11 **4.1. Phylogenetic Relationships**

12 The topologies suggested by the multi-locus ML and the time-scaled BI analyses showed consistency in the clustering of the genera Morimus (Phrissomini syn. of Lamiini), 13 Dorcadion (Dorcadionini), Monochamus (Monochamini) and Batocera (Batocerini) 14 15 (Figure 1, Figure S2). Single-locus COI-based NJ and ML trees analyses provided a 16 broader aspect due to covering more taxa, and clustered Dorcadion and Iberodorcadion 17 (Dorcadionini); Imantocera (Gnomini), Batocera and Apriona (Batocerini) with genera Lamia, Morimus, Paraleprodera, Blepephaeus and Peblephaeus at (Supplementary 18 Figure S1, Supplementary Figure S2). This propinquity has long been recognized and 19 suggested merging all tribes which have the guise of Monochamini by Breuning (1961) 20 21 and supported by the following studies (Ohbayashi et al., 2009; Toki and Kubota, 2010; 22 Gorring, 2019; de Santana Souza et al., 2020). Additionally, de Santana Souza et al. 23 (2020) perceived Dorcadionini as the closest relative of Lamiini, despite its apparent difference, which is most probably due to only adaptive characters (Löbl and Smetana, 24

1 2010). The close kinship of Lamiini and Dorcadionini was also supported by Giannoulis 2 et al. (2020); however, their results were dissimilar to other studies due to the placement 3 of *Phytoecia* (Phytoeciini) at the base of the Lamiini+Dorcadionini branch together with 4 Monochamus (Monochamini). Our results were conformable with the results of de 5 Ashman et al. (2022) and de Santana Souza et al. (2020) on the station of Monochamus 6 and *Batocera* closely. Ren et al. (2021) also supported the sisterships of Batocerini and 7 Lamiini. Contemplating the results of the present study and the previous studies 8 mentioned above, the expectations of Lacordaire (1869) and Pascoe (1866) (as per de 9 Santana Souza et al., 2020) regarding the close relationship between Batocerini and 10 Monochamini seem to be plausible, and Dorcadionini, Gnomini, and Monochamini (at 11 least in terms of Monochamus) should be revised.

12 Mesosini split from Lamiini and close relatives mentioned above by the absence of a sharp tubercle or spine at the lateral margin of the pronotum and a simple mesotibia (Bílý 13 and Mehl, 1989; Bense, 1995). However, for both tribes, the first antennal segment has a 14 15 rounded apex with a carina, which separates them from Saperdini (Bílý and Mehl, 1989). 16 The proximity of Mesosini to the cluster of Lamiini has been shown in the phylogenetic 17 trees of de Santana Souza et al. (2020) and is supported by our results. (Figure 1, Figure 2, Supplementary Figure S1, Supplementary Figure S2). However, unlike de Santana 18 19 Souza et al. (2020), Mesosa was not in the same subcluster as Saperda (Saperdini) in our 20 phylogenetic trees. Therefore, the apex structure of the first antennal segment might be 21 evaluated as a mark for the synapomorphy of Mesosini and Lamiini.

Phrynetini, represented by *P. leprosa*, was nested in Pogonocherini in our phylogenetic
trees, while it was at the base of the Acanthoderini, according to de Santana Souza et al.
(2020)'s results. This tribe is mostly occurring Afrotropic and Indomalaya regions, and its

closely related taxa were possibly not included in analyses; thus, it would not be accurate
 to conclude its relationships.

Our results support the closeness of *Pogonocherus* and *Exocentrus* shown by de Santana Souza et al. (2020) and Ashman et al. (2022). On the other hand, considering our *COI*based ML tree (Supplementary Figure S2), clusterings of *Ecyrus* and *Exocentrus* of Pogonocherini with *Rosalba* sp. (Apomecynini) and *Bactriola* sp. (Forsteriini), respectively might be a sign of a need for questioning monophyly of Forsteriini by extensive sampling and broader genetic data, in addition to 12 tribes and Apomecynini discussed by de Santana Souza et al. (2020)

The tribes Acanthocinini and Acanthoderini, which share some morphological traits such as antennae lacking long erect hairs and laterally closed joint sockets of middle coxa (Bílý and Mehl, 1989; Bense, 1995), were sister clades in our time-scaled-BI and ML analyses, *A. clavipes*, the sole representative of the Acanthoderini, stated at the base of Acanthocinini, while it was clustered with *P. leprosa* from Phrynetini in de Santana Souza et al. (2020).

Agapanthiini, the 12-segmented tribe of the subfamily, was not monophyletic at the *COI*based NJ and ML gene trees of the present study; *C. filum* was clustered with the genus *Agapanthia*. In contrast, *Hippopsis* sp. from this tribe was clustered out of this group,
with *O. singularis* from the tribe Acanthomerosternoplini (Supplementary Figure S1).
Agapanthiini seems polyphyletic at ML tree of de Santana Souza (2020) et al., in terms of *Hippopsis* sp., also.

The closeness of Obereini, Phytoeciini, and Saperdini was shown by de Santana Souza et al. (2020), who used the genera *Oberea* from the tribe Obereini and *Mecas* (*Dylobolus*) and *Phytoecia* from Phytoeciini, and *Glenea, Paraglenea*, and *Saperda* from Saperdini

the present study's *COI*-based NJ and ML gene trees supported their results. The Saperdini group included *Eutetrapha, Glenea, Mecas, Saperda, Stenostola, Thyestilla,* and *Phytoecia* (Supplementary Figure S1, Supplementary Figure S1). Same as Ren et al. (2021), and Ashman et al. (2022), our findings support the suggestion of de Santana Souza et al. (2020) that the tribe Phytoeciini should also be a synonym of Saperdini or all members should be evaluated separately.

7 **4.2.** Diversification Times

8 Initial evolutionary history studies rely on genetic data to estimate the emergence of 9 cerambycids from the Late Cretaceous (Gómez-Zurita et al., 2007) to the Early 10 Cretaceous (Wang et al., 2014; Zhang et al., 2018), while recent studies, which were 11 conducted with broader samplings and genetic data, have pointed out earlier emergence 12 time, around late Jurassic to early Cretaceous (Nie et al., 2021; Ashman et al., 2022). Our time-scaled BI analysis dated the crown age ~153 Mya, at Late Jurassic to Early 13 Cretaceous, compatible with the emergence times presented in previous studies (Farrell, 14 15 1998; Wang et al., 2014; Yu et al., 2015; Zhang et al., 2018; McKenna et al., 2019; Nie et 16 al., 2020; Ashman et al., 2022). Ashman et al. (2022) and Nie et al. (2021), whose time 17 estimation studies cover broader laminin samples, have conflicts in some emergence times of MRCA of tribes, probably due to differences between their genomic data, 18 19 calibration points, and the taxa included in the analyses. Also, our estimations resulted in 20 some discordances due to the same reasons. All three of us are probably missing the most 21 major branching events due to the scarcity of the taxa.

According to the chronogram of Ashman et al. (2022), MRCA of the subfamily Lamiinae emerged at ~105 Mya; *Acanthocinus griseus* (Acanthocinini) split from the MRCA of Lamiinae and stated at the base of the Lamiinae clade. According to our chronogram, the

1 emergence of the MRCA of the subfamily ~128 Mya was approximately 50 Mya earlier 2 than the MRCA of Acanthocinini and Acanthoderini, which emerged ~78 Mya at the late Cretaceous. The split of A. griseus and A. aedilis was dated ~23 Mya, earlier than the 3 4 oldest known fossil species of the genus Acanthocinus schmidti Schmidt 1967 3.6 to 5 2.588 Mya from Pliocene of Germany (Gersdorf, 1976). Other fossil records Astynomus 6 tertiarius Kolbe 1888 and Kallyntrosternidius bucarensis Vitali, 2009 of Acanthocinini 7 address an earlier date (15.97 to 13.65 Mya) Miocene of Germany and 20.43 to 13.65 8 Mya Miocene of Dominican Republic (Vitali, 2009), respectively. The fossil records of Acanthoderini, Acanthoderus lepidus Heer 1865, Acanthoderes phrixi Heer 1847 and 9 Acanthoderus sepultus Heer 1865, are known from the Miocene of Croatia and Germany 10 11 12.7 to 11.6 Mya (Heer, 1879). However, any study that mentions fossil traces of a 12 common ancestor of Acanthocinini and Acanthoderini has yet to be encountered.

The MRCA of the Lamiini and close relatives, which include *Monochamus* and *Batocera*, 13 appeared at the Eocene epoch of Paleogene, ~52.53 Mya according to our chronogram, 14 15 relatively consistent, but a bit earlier than the chronogram of Ashman et al. (2022) and 16 Nie et al. (2021) which point to ~63 Mya and ~55 Mya, respectively. This is the youngest 17 clade compared to the other tribes included in this study (Figure 2). The fossil records of Lamiini, Lamia antiqua Heer 1879, from Miocene of Germany (12.7 to 11.6 Mya) (Heer, 18 19 1879), Dorcadion bachense Handschin 1944 from Oligocene of France (28.4 to 23.03 Mya) (Handschin, 1944), and Dorcadion emeritum von Heyden 1862 from Oligocene of 20 21 Germany (28.4 to 23.03 Mya) (Von Heyden, 1862) are roughly concordant with our 22 estimation, which dated splitting of *Dorcadion* from its last common ancestor genus 23 Morimus ~26 Mya. The speciation event within the genus Dorcadion was 14.34 Mya in

1 our estimation, earlier than the chronogram of Dascălu et al. (2022), who dated the 2 beginning of speciation within the genera *Dorcadion* was around 9.8 Mya. 3 The emergence time of *Mesosa* was ~ 67 Mya according to our chronogram, but ~ 107 4 Mya according to Nie et al. (2020). All fossil records of Mesosa Latreille 1829 (syn. 5 Sinocalosoma Hong 1983) were from the Miocene of China and the Oligocene of 6 Germany (28.4 to 11.608 Mya), (Hong 1983, Hong and Wang 1986). 7 Considering our chronogram, the MRCA of four species of Agapanthia appeared about 8 55 Mya in Paleogene (Figure 2), while the MRCA of two Agapanthia daurica individuals 9 dated 51.79 Mya in Nie at el. (2021). However, we could not access any fossil record for 10 this genus. The MRCA of Pogonocherus and Exocentrus emerged around 65 Mya at the edge of the 11 12 Cretaceous and Paleogene, according to the chronogram of Ashman et al. (2022), while it is 37 Mya around mid-Paleogene, according to the hypothesized chronogram in the 13 present study (Figure 2). The oldest fossil of Pogonocherus, Pogonocherus jaekeli Zang 14 15 1905, is from Baltic Amber, 37.2 to 33.9 Mya (Vitali, 2009). 16 We estimated the MRCA of Saperdini and Phytoeciini as 65 million years old, which the 17 latter might be a synonym of the preceding in addition to Obereini (de Santana Souza et 18 al. 2020). Ashman et al. (2022) estimated the MRCA of *Phytoecia* and *Oberea* 36 Mya. 19 The oldest fossil of the genus Saperda is Saperda caroli Vitali 2015, from Uintan of 20 Colorado, America (46.2 - 40.4 Mya) and the oldest fossil from Eurasia is Saperda 21 densipunctata Théobald 1937, from Oligocene of Germany, 33.9 to 28.4 Mya (Vitali, 22 2015). The oldest Obereini fossil is the youngest in the group, Oberea praemortua von 23 Heyden 1862, and from the Miocene of Germany, 15.97 to 11.608 Mya (Von Heyden,

24 1862).

According to our time estimation of the intrageneric diversification of the taxa involved
in the analysis occurred during Cenozoic, mostly in Neogene, concurred with Ashman et
al. (2022). Diversification of the members of *Agapanthia* seems to be occurred during
Paleogene ~55 Mya, earlier than *Phytoecia* and *Dorcadion* members, ~23 Mya and ~14
Mya, respectively, during Neogene.

6 What our study reveals is the Lamiinae specimens from East of Marmara Basin, Türkiye, 7 were closely related to European conspecifics and congeners. And, relying on our 8 samples, we stand by the side of de Santana Souza et al. (2020) in supporting the 9 hypothesis of uniting the tribes Dorcadionini, Gnomini, Monochamini (in terms of 10 Monochamus) and the current Lamiini under a single tribe Lamiini. Considering all 11 recent studies and the present study, the emergence time of the subfamily Lamiinae is 12 Jurassic to early Cretaceous, and the extant species included in this study presently occur in the East of Marmara Basin that emerged during the Neogene period. 13

14 Acknowledgements

We thank Dr Burcu Şabanoğlu Şimşek and Dr Hüseyin Özdikmen for their contributions to morphological identification, Dr Şener Atak and Osman Atak for their sampling efforts and Saadet Şirin Coşkun for visualisation of the figures. This study is a part of PhD thesis of Havva Kübra Soydabaş Ayoub and funded by the Scientific Research Foundation of Kocaeli University, grant number BAP 139-2018.

20 References

Ashman LG, Shin S., Zwick A., Ślipiński A., McKenna DD. (2022). The first phylogeny
 of Australasian Lamiinae longhorn beetles (Coleoptera: Cerambycidae) reveals poor
 tribal classification and a complex biogeographic history. Systematic Entomology, 47(1):

24 213-230. https://doi.org/10.1111/syen.12526

1	Atak Ş,	Uçkan	F,	Soydabaş-Ayoub	ΗK	(2021).	A	faunistic	study	on	Cerambycidae
---	---------	-------	----	----------------	----	---------	---	-----------	-------	----	--------------

- 2 (Coleoptera) of Kocaeli province (Turkey). Plant Protection Bulletin, 61(2): 33-43.
- 3 https://doi.org/10.16955/bitkorb.796414
- 4 Bense U (1995). Longhorn beetles: illustrated key to the Cerambycidae and Vesperidae
- 5 of Europe. Germany: Margraf Verlag.
- 6 Bílý S, Mehl O (1989). Longhorn Beetles (Coleoptera, Cerambycidae) of Fennoscandia
- 7 and Denmark, Netherlands: Brill.
- 8 Breuning S (1951). Revision du genre Phytoecia Muls. (Coleoptera Cerambycidae).
- 9 Entomologische Arbeiten aus dem Museum G. Frey 2: 1-103 (in French).
- 10 Breuning SV (1961). Catalogue des Lamiaires du Monde (Col., Cerambycidae). Lief
- 11 Tutzing bei München, Germany: Museum G. Frey (in French).
- 12 Cockerell TDA (1927). Fossil insects in the British Museum. The Annals and Magazine
- 13 of Natural History, Ninth Series 20: 585-594.
- Çakmak YE, Ayoub HKS, Uckan F (2019). Size divergence of *Rhagium inquisitor*:
 sexual similarity versus environmental variability. Feb-Fresenius Environmental Bulletin
 28: 7593-7602.
- 17 Çakmak YE, Soydabaş-Ayoub HK, Uckan, F (2020) A preliminary phylogenetic analysis
- 18 of ribbed-pine-borer (Rhagium inquisitor) based on mitochondrial COI sequences.
- 19 Journal of Asia-Pacific Entomology 23(3): 809-815. doi.org/10.1016/j.aspen.2020.06.008
- 20 Dascălu MM, Caba FG, Fusu L (2022). DNA barcoding in Dorcadionini (Coleoptera,
- 21 Cerambycidae) uncovers mitochondrial-morphological discordance and the hybridogenic

origin of several subspecies. Organisms Diversity & Evolution, 1-25.
 https://doi.org/10.1007/s13127-021-00531-x

de Santana Souza D, Marinoni L, Monné ML, Gómez-Zurita J (2020). Molecular
phylogenetic assessment of the tribal classification of Lamiinae (Coleoptera:
Cerambycidae). Molecular Phylogenetics and Evolution, 145: 106736.
https://doi.org/10.1016/j.ympev.2020.106736

7 Drummond AJ, Rambaut A (2007). BEAST: Bayesian evolutionary analysis by sampling

8 trees. BMC Evolutionary Biology, 7(1): 1-8. https://doi.org/10.1186/1471-2148-7-214

9 Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006). Relaxed phylogenetics and
10 dating with confidence. PLoS Biology, 4(5): e88.
11 https://doi.org/10.1371/journal.pbio.0040088

Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high
throughput. Nucleic Acids Research, 32(5): 1792-1797.
https://doi.org/10.1093/nar/gkh340

15Farrell BD (1998). " Inordinate Fondness" explained: why are there So many16beetles?. Science, 281(5376),555-559.

17 https://www.science.org/doi/10.1126/science.281.5376.555

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994). DNA primers for
amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan
invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.

1	Gernhard T (2008). The conditioned reconstructed process. Journal of Theoretical
2	Biology, 253(4): 769-778. https://doi.org/10.1016/j.jtbi.2008.04.005
3	Gersdorf E (1976). Dritter Beitrag über Käfer (Coleoptera) aus dem Jungtertiär von
4	Willershausen, Bl. Northeim 4226. Geologisches Jahrbuch A, 36: 103-145 (in German).
5	Giannoulis T, Dutrillaux AM, Sarri C, Mamuris Z, Dutrillaux B (2020). Phylogenetic
6	relationships between genera Dorcadion, Lamia, Morimus, Herophila and some other
7	Lamiinae (Coleoptera: Cerambycidae) based on chromosome and CO1 gene sequence
8	comparison. Bulletin of Entomological Research, 110(3): 321-327.
9	https://doi.org/10.1017/S0007485319000737
10	Gómez-Zurita J, Hunt T, Kopliku F, Vogler AP (2007). Recalibrated tree of leaf beetles
11	(Chrysomelidae) indicates independent diversification of angiosperms and their insect
12	herbivores. PLoS ONE 2(4):e360. https://doi.org/10.1371/journal.pone.0000360
13	Gorring PS (2019). Gene to Genus: Systematics and Population Dynamics in Lamiini
14	Beetles (Coleoptera: Cerambycidae) With Focus on Monochamus Dejean. PhD, Harvard
15	University, USA.
16	Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W. et al. (2010) New
17	algorithms and methods to estimate maximum-likelihood phylogenies: assessing the

18 performance of **PhyML** 3.0. Systematic Biology, 59(3): 307-321. https://doi.org/10.1093/sysbio/syq010 19

Haddad S, Shin S, Lemmon AR, Lemmon EM, Svacha P et al. (2018). Anchored hybrid 20 enrichment provides new insights into the phylogeny and evolution of longhorned beetles 21 (Cerambycidae). Systematic Entomology, 43: 68-89. https://doi.org/10.1111/syen.12257 22

1	Handschin E (1944). Insekten aus den Phosphoriten des Quercy. Schweizerische
2	Paläontologische Abhandlungen 64(4): 1-23 (in German).
3	Heer O (1879). Die Urwelt der Schweiz 2nd ed. Germany: F. Schulthess (in German).
4	Hong YC (1983). Fossil insects in the diatoms of Shanwang. Bulletin of the Tianjin
5	Institute of Geology and Mineral Resources 8: 1-15.
6	Hong YC, Wang WL (1986). Miocene Coleoptera (Insecta) of Shanwang, Shandong
7	Province, China. Memoirs of Beijing Natural History Museum 38: 1-19.
8	Hunt T, Bergsten J, Levkanicova Z, Papadopoulou A, John OS et al. (2007). A
9	comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation.
10	Science, 318(5858): 1913-1916. https://doi.org/10.1126/science.1146954
11	Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M et al. (2012) Geneious Basic:
12	an integrated and extendable desktop software platform for the organization and analysis
13	of sequence data. Bioinformatics, 28(12): 1647-1649.
14	https://doi.org/10.1093/bioinformatics/bts199
15	Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new
16	methods for selecting partitioned models of evolution for molecular and morphological
17	phylogenetic analyses. Molecular biology and evolution 34(3): 772-773.
18	https://doi.org/10. 1093/molbev/msw260.
19	Linsley EG (1959). Ecology of Cerambycidae. Annual Review of Entomology, 4(1): 99-

20 138.

Löbl I, & Smetana A (2010). Catalogue of Palaearctic Coleoptera. Volume 6:
 Chrysomeloidea. Stenstrup, Denmark: Apollo Books.

Marvaldi AE, Duckett CN, Kjer KM, Gillespie JJ (2009) Structural alignment of 18S and
28S rDNA sequences provides insights into phylogeny of Phytophaga (Coleoptera:
Curculionoidea and Chrysomeloidea). Zoologica Scripta 38(1): 63-77.
https://doi.org/10.1111/j.1463-6409.2008.00360.x

McKenna DD, Wild AL, Kanda K, Bellamy CL, Beutel RG et al. (2015). The beetle tree
of life reveals that Coleoptera survived end Permian mass extinction to diversify during
the Cretaceous terrestrial revolution. Systematic Entomology, 40(4): 835-880.
https://doi.org/10.1111/syen.12132

McKenna DD, Shin S, Ahrens D, Balke M, Beza-Beza C et al. (2019). The evolution and
genomic basis of beetle diversity. Proceedings of the National Academy of
Sciences, 116(49): 24729-24737. https://doi.org/10.1073/pnas.1909655116

Monné ML, Monné MA, Wang Q (2017) General Morphology, Classification and
Biology of Cerambycidae. In Wang, Q (editor) Cerambycidae of the World. Biology and
Pest Management, USA: Taylor and Francis, CRC Press, pp. 1-70.

17 Napp DS (1994). Phylogenetic relationships among the subfamilies of Cerambycidae

- 18 (Coleoptera, Chrysomeloidea). Revista Brasileira de Entomologia, 38(2): 265-419.
- Nie R, Vogler AP, Yang XK, Lin M (2021). Higher level phylogeny of longhorn beetles
 (Coleoptera: Chrysomeloidea) inferred from mitochondrial genomes. Systematic
- 21 Entomology, 46(1): 56-70. https://doi.org/ 10.1111/syen.12447

1	Ohbayashi N, Ogawa J, Su ZH (2009). Phylogenetic Analysis of the Lamiine Genus
2	Anoplophora and Its Relatives (Coleoptera, Cerambycidae) Based on the Mitochondrial
3	COI Gene, Special Bulletin Japanese Society of Coleopterology, 7: 309-324.
4	Özdikmen H (2013) Turkish Agapanthiini Mulsant, 1839 with identification keys
5	(Coleoptera: Lamiinae), Munis Entomology & Zoology, 8 (1): 9-40.
6	Piton L, Théobald N (1937). Les lignites et schistes bitumineux de Menat (Puy-de-
7	Dôme). II: Les insectes fossiles de Menat. Revue des Sciences Naturelles
8	d'Auvergne 3(2): 76-88 (In French).
9	Rambaut A (2014). FigTree v1. 4.2, a graphical viewer of phylogenetic trees. Available
10	from http://tree. bio. ed. ac. uk/software/figtree/
11	Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018). Posterior
12	summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5):
13	901-904. https://doi.org/10.1093/sysbio/syy032s
14	Ren Y, Lu H, Chen L, Sabatelli S, Wang C et al. (2021). Comparative Mitogenomic
15	Analysis of Two Longhorn Beetles (Coleoptera: Cerambycidae: Lamiinae) with
16	Preliminary Investigation into Phylogenetic Relationships of Tribes of Lamiinae. Insects,
17	12(9): 820. https://doi.org/10.3390/insects12090820
18	Sama G (2002). Atlas of the Cerambycidae of Europe and Mediterranean area. Vol 1:

19 Northern, Western, Central and Eastern Europe. Czech Republic: Kabourek.

1	Sonnenberg R, Nolte AW, Tautz D (2007). An evaluation of LSU rDNA D1-D2
2	sequences for their use in species identification. Frontiers in Zoology, 4: 1-12.
3	https://doi.org/10.1186/1742-9994-4-6
4	Soydabaş Ayoub HK (2021). Nüklear ve Mitokondriyal DNA Dizileri Kullanılarak

Kocaeli Ilindeki Cerambycidae (Coleoptera) Türlerinin Filogenetik Analizi. PhD, Kocaeli
University, Türkiye (In Turkish).

Soydabaş-Ayoub HK, Uçkan F, Atak Ş, Şimşek B, İbiş O (2022). Assessment of the
phylogenetic relationships within the spondylidine branch of Spondylidinae (Coleoptera,
Cerambycidae). Insect Systematics & Evolution 54(3): 281-311.
https://doi.org/10.1163/1876312X-bja10042

Švácha P, Lawrence JF (2014). Cerambycidae Latreille, 1802. In Leschen RAB, Beutel
RG (editors). Handbook of Zoology, Arthropoda: Insecta; Coleoptera, Beetles, Volume 3:
Morphology and Systematics (Phytophaga) 1st ed. Berlin, Germany: Walter De Gruyter
pp. 147-151.

Toki W, Kubota K (2010). Molecular phylogeny based on mitochondrial genes and
evolution of host plant use in the long-horned beetle tribe Lamiini (Coleoptera:
Cerambycidae) in Japan. Environmental Entomology 39(4): 1336-1343.
https://doi.org/10.1603/EN09347

19 Villiers A (1978). Faune des Coléoptères De France. I. Cerambycida, Encyclopedie
20 Entomologique, Lechevalier: Publications de la Société Linnéenne de Lyon.

Vitali F (2009). Two new longhorned beetles from Dominican amber (Coleoptera,
Cerambycidae). Denisia 26: 223-230.

1	Vitali F (2015). Saperda caroli n. sp., a new fossil species from Early Eocene of
2	Colorado, with taxonomic remarks on its extinct congeners (Coleoptera, Cerambycidae).
3	Les Cahiers Magellanes NS 17: 20-28.
4	Von Heyden C (1862). Gliederthiere aus der Braunkohle des Niederrhein's, der Wetterau
5	und der Röhn. Palaeontographica 10(2): 62-82.
6	Wallin H, Nylander U, Kvamme T (2009). Two sibling species of Leiopus Audinet-
7	Serville, 1835 (Coleoptera: Cerambycidae) from Europe: L. nebulosus (Linnaeus, 1758)
8	and <i>L. linnei</i> sp. nov. Zootaxa 2010(1): 31-45.
9	Wang B, Ma J, McKenna DD, Yan EV, Zhang H et al. (2014). The earliest known
10	longhorn beetle (Cerambycidae: Prioninae) and implications for the early evolution of
11	Chrysomeloidea. Journal of Systematic Palaeontology 12(5): 565-574. https://doi:
12	0.1080/14772019. 2013.806602
13	Wei ZH, Yin XM, An SH, Su LJ, Li J et al. (2014). Molecular Phylogenetic Study of the
14	Higher Taxa of the Superfamily Cerambycoidea (Insecta: Coleoptera) Based on the

16 710-720.

15

Yoon HJ, Bae JS, Kim I, Jin BR, Mah YI et al. (2001) A phylogenetic study in some
long-horned beetles (Coleoptera: Cerambycidae) using mitochondrial *COI* gene and *16S rRNA* sequences. International Journal of Industrial Entomology 2(1): 37-53.

Combined Analysis of Ribosomal DNA Sequences. Acta Entomologica Sinica 57(6):

Yu Y, Ślipiński A, Reid C, Shih C, Pang H et al. (2015). A new longhorn beetle
(Coleoptera: Cerambycidae) from the Early Cretaceous Jehol Biota of western Liaoning
in China. Cretaceous Research 52: 453-460. https://doi.org/10.1016/j.cretres.2014.02.019

- 1 Zhang SQ, Che LH, Li Y, Liang D, Pang H et al. (2018). Evolutionary History of
- 2 Coleoptera revealed by extensive sampling of genes and species. Nature communications
- 3 9(1): 1-11. https://doi.org/10.1038/s41467-017-02644-4

4 Figures



- 5 Figure 1. Phylogenetic relationships recovered by maximum likelihood (ML) analysis of
- 6 spliced datasets of mitochondrial COI and 16S rRNA and nuclear 28S rRNA gene regions.
- 7 Bootstrap supports are indicated beside nodes.
- 8



Figure 2. Divergence times recovered by Bayesian analysis of spliced datasets of mitochondrial COI and *16S rRNA* and nuclear *28S rRNA* gene regions. The brown bars correspond to the 95% highest posterior density (HPD) interval; the mean ages are above, and the posterior probabilities are below each node; the scale bars indicate the time in million years; Q: Quaternary.

1 Supplementary Materials

Supplementary Figure S1: The Neighbor-Joining tree of the subfamily Lamiinae 2 3 comprises a representative haplotype for each available species from each zoogeographic 4 region based on mitochondrial COI sequences (658 bp) obtained in this study and 5 retrieved from the BOLD taxonomy archive 6 Supplementary Figure S2: The Maximum Likelihood tree of the subfamily Lamiinae 7 comprises a representative haplotype for each available species from each zoogeographic 8 region based on mitochondrial COI sequences (658 bp) obtained in this study and 9 retrieved from the BOLD taxonomy archive Supplementary Table S1. Binomial names, voucher codes, sampling coordinates, 10 localities and GenBank accession numbers of the specimens sampled in this study 11 12 Supplementary Table S2: Identity numbers, binomial names and sampling localities of mitochondrial COI gene region sequences of subfamily Lamiinae, which were retrieved 13 from the BOLD taxonomy database. 14

15 **Conflicts of Interest**

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

19 Data Availability Statement

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. The sequences produced in this study are available in GenBank under the accession numbers OP279135-OP279183, OP279535-OP279581 and OP279486-OP279532.



