Time-scaled phylogenetic analysis of the Lamiini, its close relatives and some other widely distributed tribes of Lamiinae (Coleoptera, Cerambycidae)

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ABSTRACT

Lamiinae is a tremendous subfamily of Cerambycidae, with around 20,000 members dispersed across continents. The knowledge of the evolutionary history of the subfamily is scarcely, and there are growing doubts about the phylogenetic relationships due to the recognised illusion caused by the convergence of the morphological characters. The present study contributes to the evolutionary history and phylogenetic relationships of the tribes Acanthocinini, Acanthoderini, Agapanthiini, Batocerini, Dorcadionini, Lamiini, Mesosini, Monochamini, Phytoeciini, Phrynetini, Pogonocherini (including Exocentrini) and Saperdini with Neighbor-Joining (NJ), Maximum Likelihood (ML) and time-scaled Bayesian analyses based on partial mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (2257 base pair alignment length). The most recent common ancestor (MRCA) of the taxa included in the analyses appeared during the Middle or Late Cretaceous, and the MRCAs of the closely related tribes emerged in Paleogene. The MRCA of Dorcadionini, Lamiini and Monochamini was younger than the common ancestors of the other close tribes. The hypothetical ML phylogram was consistent with the Bayesian chronogram in the proximity of Batocerini to Lamiini, Acanthocinini to Acanthoderini, Phyretrini to Pogonocherini, and Phytoeciini to Saperdini, in addition to the affiliation of Lamiini, Dorcadionini and Monochamini. At the *COI*-based NJ and ML gene trees, *Paraleprodera* and *Lamia* (Lamiini) were sisters to *Imantocera* (Gnomini), *Oberea* (Obereini) to *Phytoecia* (Phytoeciini), and *Hippopsis* (Agapanthiini) to *Omosarotes* (Acanthomerosternoplini). The present results support Dorcadionini Gnomini and Monochamini as synonyms of Lamiini; and Obereini and Phytoeciini of Saperdini. We suggest that the emergence of the living tribes included in this study was during Paleogene, and the intrageneric diversifications occurred in Cenozoic, mostly during Neogene.

Keywords: Common ancestors, Cretaceous, Flat-faced longhorns, Intrageneric diversification times, Molecular clock, Neogene, Paleogene.

INTRODUCTION

Lamiinae, the 'flat-faced longhorns', constitute over half of the long-horned beetles Cerambycidae (Coleoptera) by more than 20,000 species (Švácha & Lawrence, 2014; Tavakilian & Chevillotte, 2015), distributed in all biogeographic realms except Antarctica (Monné et al., 2017). The evolutionary relationships of the subfamily have been in the limelight of Coleopterists, especially in the last decade. The studies rely on either morphological or molecular synapomorphic characters or both (Ashman et al., 2022; Haddad et al., 2018; Napp, 1994; Nie et al., 2021; Raje et al., 2016; Švácha & Lawrence, 2014, Wei et al., 2014; Wu et al., 2017), have reached almost a complete unanimity on the monophyly of the subfamily. However, the morphological and molecular studies conflict about who the sister clade of the subfamily is. The recent molecular phylogenetic analyses (Gómez-Zurita et al., 2007, 2008; Haddad et al., 2018, 2021; Nie et al., 2021; Marvaldi et al., 2009) have arrived at concurrence that Spondylidinae is the sister clade of Lamiinae and have rejected the assumption of the previous morphological studies (Linsley, 1961; Napp, 1994; Villiers, 1978), which had offered Cerambycinae.

The phylogenetic picture becomes blurrier towards lower taxonomic levels. Phyletic statuses of the tribes, especially of the species-rich ones distributed across continents, are doubtful due to the evolutionary convergence of some morphological characters (de Santana Souza et al., 2020). In recent years, some studies have taken initial steps to resolve the tribal-level relationships of Lamiinae. de Santana Souza et al. (2020) studied 46 tribes by analysing the fragments of two mitochondrial and three nuclear markers and inferred that some tribes such as Astathini, Batocerini, Ceroplesini, Colobotheini, Compsosomatini, Dorcadionini, Lamiini, Mesosini, Obereini and Polyrhaphidini are monophyletic; but, Acanthocinini, Acanthoderini, Agapanthiini,

Apomecynini, Desmiphorini, Dorcaschematini, Enicodini, Hemilophini, Monochamini, Onciderini, Parmenini, Phytoeciini, Pogonocherini, Pteropliini and Saperdini are not. Ren et al. (2021) analysed 13 protein-coding genes of mitogenomes of representatives of 12 tribes. They suggested the sisterships of Ceroplesini and Agapanthiini, Mesosini and Pteropliini, Saperdini and Phytoeciini, Batocerini and Lamiini, and Apomecynini and Acanthocinini.

The remarkable studies contributing evolutionary history of the subfamily mainly focused on higher taxa. Gómez-Zurita et al. (2007), relying on three partial ribosomal gene regions of 167 chrysomelid taxa and molecular clock calibration based on the oldest fossils of Cassidinae and Timarchini, suggested that the origin node of the cerambycids points to the Late Cretaceous around 80 mya. Then the divergence timing was recalibrated by Wang et al. (2013) due to a discovery of the known earliest cerambycid fossil, *Cretoprionus liutiaogouensis* Wang, Ma, McKenna, Yan, Zhang and Jarzembowski, 2013 (Prioninae) from the Early Cretaceous of China. According to their estimation, the origin of modern Cerambycidae was the Late Triassic (about 210 mya), older than the conjecture of Gómez-Zurita et al. (2007), and all subfamilies arose by the mid-Cretaceous. Then, again, the emergence time of the family was shifted to a further date, to the beginning of the Early Cretaceous around 145 (mya) by Zhang et al. (2018), based on 95 protein-coding genes in 373 taxa of Coleoptera. The study by Nie et al. in 2021, based on 151 mitochondrial genomes covering all families and 29 subfamilies of Chrysomeloidea, dated the emergence of the common ancestor of Cerambycidae *s. l.*, Megalopodidae and Orsodacnidae around 150.9 Mya, the common ancestor of the Spondylidinae and Lamiinae at 149.03 mya, and the ancestor of the crown group of the subfamily Lamiinae at 132.0 mya.

The first study directly dealing with the evolutionary history of Lamiinae was conducted by Ashman et al. (2022) to unveil the biogeographic origin of Australasian Lamiinae. According to their estimation, the common ancestor of Cerambycidae appeared in the Late Jurassic to Early Cretaceous 129 Ma, and with an alternate fossil calibration ∼188 mya, without resulting change in branch lengths within Lamiinae, arose from the Late Cretaceous to the Palaeocene 64-76 mya.

Up to date, any study dealing with divergence times of the tribes of the subfamily Lamiinae, except Australasians, has not been conducted yet. The present study aims to contribute to resolving the evolutionary history of the tribes Acanthocinini, Acanthoderini, Agapanthiini, Monochamini, Phytoeciini, Pogonocherini, and Saperdini with samples from the Marmara Basin, Turkey, which is an intersection between Balkan Peninsula and Asia Minor, using partial mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions.

MATERIALS AND METHODS

Fieldworks and Morphological Identification

Specimens were collected from timber yards, wood processing plants, suburban areas and forests between 2016 and 2019 in the East of Marmara Basin, Turkey (Table 1), a remarkable region for biodiversity (Atak et al., 2021; Çakmak et al., 2019). Ethanol and α-pinene tubes were used within three funnel traps. Insect nets were used for flower-visiting species. *Phryneta leprosa* (Castilla-borer) was intercepted in Derince port on timber imported from Cameroon, and *Batocera rufomaculata* (tropical fig-borer) was caught coincidentally in Diyarbakır, Turkey. Morphological identifications were carried

out with the guidance of Bense (1995), Bílý and Mehl (1989), Breuning (1951), Harde (1966), Özdikmen (2013), Plavilstshikov (1930), Rossa, Goczał and Tofilski (2017), Sama (2002), Wallin, Nylander and Kvamme (2009), and Zamoroka and Kapelyukh (2012) under a stereomicroscope (Olympus SZ51, Japan) enhanced by Olympus 110AL2X-2 WD38 auxiliary macro lens. Specimens were stored in 99% ethanol at -20 ºC pending DNA extraction. S

DNA extraction, PCR and Sequencing

Depending on the specimen sizes, muscle tissues of coxa or antennae were shredded on disposable sterile slides by disposable scalpels. Then transferred into a microcentrifuge tube including the lysis buffer (8 mM dithiothreitol (DTT), 2% sodium dodecyl sulphate (SDS), 100 mM NaCl, 3 mM $CaCl_2$, dissolved in 100 mM Tris buffer (pH 8) and 200 µg/mL proteinase K) (Soydabaş-Ayoub et al., in review) and kept in Eppendorf Thermomixer 5350 heater at 37°C until whole tissue whittle down. Genomic DNA was isolated by following the phenol:chloroform:isoamyl alcohol (25:24:1) protocol (Sambrook & Russell, 2006) and precipitated by ethanol (Green & Sambrook, 2016).

Polymerase chain reaction (PCR) products were amplified in a total volume of 25µL with QuickLoad® Taq 2X Master Mix (New England Biolabs Inc., catalogue number M0271L) and 0.2 µM primer for each pair, with the addition of 0.06 mg/mL bovine serum albumin as an enhancer to avoid PCR inhibition by melanin (Giambernardi et al., 1998). The primer pairs HCO2198–LCO1490 (Folmer et al., 1994) and HCO2198-JJ-LCO1490-JJ (Astrin & Stüben, 2008) for the mitochondrial cytochrome *c* oxidase I (*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 rev2: 1048-1067 (Sonnenberg et al., 2007)

for nuclear *28S rRNA* gene regions were used in amplification. Mitochondrial *COI* and *16S RNA* gene regions were amplified by using the same thermocycling parameters as 1 min at 95 °C, five cycles of 30 s at 95 °C, 1 min 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 min at 72 °C (Çakmak et al., 2020). The thermocycling parameters used for amplification of the nuclear 28S rRNA gene region were 1 min at 95 °C, four cycles of 30 s at 95 °C, 1 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 72 °C and 10 min at 72 °C (Soydabaş-Ayoub et al., under review). ExoSAP-IT™ Clean-Up Reagent was used for purification according to manufacturer instructions. Sequencing was performed by ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) at Macrogen Holland Laboratory in both directions.

Datasets

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. The heterozygote bases in nuclear gene sequences were named based on the IUPAC (International Union of Pure and Applied Chemistry) base nomenclature code system. A total of 143 obtained sequences were deposited in GenBank under the accession numbers OP279135-OP279183, OP279535-OP279581 and OP279486-OP279532 for mitochondrial *COI*, *16S rRNA* and nuclear *28S rRNA* gene regions, respectively (Table 1).

Table 1. Binomial names, voucher codes, sampling coordinates, localities and GenBank accession numbers of the specimens sampled from the East of Marmara Basin, Turkey

Tribe	Species	Voucher ID	Coordinate	Locality	COI	16S rRNA	28S rRNA
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *aedilis* (Linnaeus, 1758)	LAAA49	40°49'33.2"N 29°29'52.0"E	Timber Yard, Gebze,	OP279161	-	-
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *aedilis* (Linnaeus, 1758)	LAAA52	40°42'52.6"N 30°03'21.1"E	Wood Processing Plant, Kartepe	OP279162	OP279556	OP279504
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG345	40°49'01.6"N 29°29'49.6"E	Wood Processing Plant, Gebze	OP279165	OP279562	OP279509
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG346	40°49'33.3"N 29°29'52.1"E	Timber Yard, Gebze	OP279166	OP279560	OP279512
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG347	40°49'04.7"N 29°29'52.7"E	Timber Yard, Gebze	OP279163	OP279561	OP279507
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG354	40°49'45.4"N 29°55'05.4"E	Forest, İzmit	OP279168	OP279559	OP279508
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG358	40°41'31.4"N 29°53'55.2"E"	Countryside, Başiskele	OP279169	-	OP279510
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG44	40°47'19.3"N 29°50'45.4"E	Forest, Derince	OP279167	OP279557	OP279506
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG68	40°49'39.8"N 29°29'47.6"E	Forest, Gebze	OP279164	-	OP279505
Acanthocinini	*Acanthocinus* (*Acanthocinus*) *griseus* (Fabricius, 1793)	LAAG69	40°49'05.6"N 29°31'08.0"E	Forest, Gebze	OP279170	OP279558	OP279511
Acanthocinini	*Leiopus* (*Leiopus*) *nebulosus* (Linnaeus, 1758)	LALN311	40°49'28.1"N 29°29'48.5"E	Timber Yard, Gebze	OP279160	OP279555	OP279519
Acanthoderini	*Aegomorphus clavipes* (Schrank, 1781)	LAAC33	40°49'30.5"N 29°30'02.3"E	Gebze Forest	-	OP279536	OP279502
Acanthoderini	*Aegomorphus clavipes* (Schrank, 1781)	LAAC361	40°49'45.1"N 29°29'50.9"E	Gebze Forest	OP279155	OP279537	OP279503
Agapanthiini	*Agapanthia* (*Epoptes*) *asphodeli* (Latreille, 1804)	LAAA30	40°41'44.1"N 29°53'50.9"E	Başiskele, Countryside	OP279144	OP279574	OP279493
Agapanthiini	*Agapanthia* (*Epoptes*) *lateralis* Ganglbauer, 1883	LAAL34	40°41'49.4"N 29°53'40.9"E	Countryside, Başiskele	OP279141	OP279575	OP279494
Agapanthiini	*Agapanthia* (*Epoptes*) *lateralis* Ganglbauer, 1883	LAAL35	40°41'53.5"N 29°53'42.0"E	Başiskele, Countryside	OP279142	OP279577	OP279496

Table 1. (continues) Binomial names, voucher codes, sampling coordinates, localities and GenBank accession numbers of the specimens sampled from the East of Marmara Basin, Turkey

Tribe	**Species**	**Voucher ID**	**Coordinate**	**Locality**	**COI**	**16S rRNA**	**28S rRNA**
Agapanthiini	*Agapanthia* (*Epoptes*) *lateralis* Ganglbauer, 1883	LAAL364	40°41'49.2"N 29°54'06.2"E	Başiskele, Countryside	OP279143	OP279576	OP279495
Agapanthiini	*Agapanthia* (*Agapanthia*) *suturalis* (Fabricius, 1787)	LAAS343	40°41'24.3"N 29°53'09.7"E	Başiskele Forest	OP279139	OP279571	OP279491
Agapanthiini	*Agapanthia* (*Agapanthia*) *suturalis* (Fabricius, 1787)	LAAS36	40°42'01.4"N 29°54'24.8"E	Başiskele, Countryside	OP279136	OP279573	OP279492
Agapanthiini	*Agapanthia* (*Agapanthia*) *suturalis* (Fabricius, 1787)	LAAS37	40°40'53.9"N 29°53'08.9"E	Başiskele, Countryside	OP279137	OP279570	
Agapanthiini	*Agapanthia* (*Agapanthia*) *suturalis* (Fabricius, 1787)	LAAS46	40°41'44.4"N 29°48'54.8"E"	Gölcük, Countryside	OP279138	OP279572	OP279490
Agapanthiini	*Agapanthia (Smaragdula) frivaldszkyi* Ganglbauer, 1884	LAAF31	40°40'24.8"N 29°50'37.1"E	Gölcük, Countryside	OP279140	OP279569	OP279489
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO303	41°07'53.0"N 30°11'55.6"E	Kandıra Forest	OP279173	OP279546	OP279529
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO304	41°07'44.9"N 30°11'57.6"E	Kandıra Forest	OP279176	OP279549	OP279526
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO306	40°49'45.1"N 29°29'50.9"E	Gebze Forest	OP279174	OP279547	OP279527
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO307	40°41'20.7"N 29°54'05.7"E	Başiskele Countryside	OP279172	OP279548	OP279525
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO308	40°49'07.8"N 29°53'54.1"E	Forest, İzmit	OP279175	OP279551	
Lamiini	*Morimus orientalis* Reitter, 1894	LLMO310	41°08'25.5"N 30°10'20.0"E	Forest, Kandıra	-	OP279550	OP279528
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG286	41°07'45.9"N 30°12'50.1"E	Forest, Kandıra	OP279148	OP279540	OP279523
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG287	40°49'28.1"N 29°29'48.5"E	Timber Yard, Gebze	OP279153	-	OP279520
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG290	40°49'33.2"N 29°29'52.0"E	Timber Yard, Gebze	-	OP279542	-
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG295	40°50'19.0"N 29°27'35.9"E	Timber Yard, Gebze	OP279154	-	-
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG298	40°49'28.1"N 29°29'48.5"E	Timber Yard, Gebze	OP279151	OP279543	-

Table 1. (continues) Binomial names, voucher codes, sampling coordinates, localities and GenBank accession numbers of the specimens sampled from the East of Marmara Basin, Turkey

Tribe	**Species**	**Voucher ID**	**Coordinate**	**Locality**	**COI**	**16S rRNA**	**28S rRNA**
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG42	40°50'19.0"N 29°27'35.9"E	Timber Yard, Gebze	OP279152	-	OP279521
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG43	40°49'58.1"N 29°28'47.4"E	Timber Yard, Gebze	OP279149	OP279539	-
Monochamini	*Monochamus* (*Monochamus*) *galloprovincialis* (Olivier, 1795)	LMMG45	40°47'19.3"N 29°50'45.4"E	Forest, Derince	OP279150	OP279541	OP279522
Dorcadionini	*Dorcadion (Cribridorcadion) septemlineatum* Waltl, 1838	LDDS341	41°07'44.9"N 30°11'57.6"E	Forest, Kandıra	OP279182	OP279553	OP279531
Dorcadionini	*Dorcadion (Cribridorcadion) septemlineatum* Waltl, 1838	LDDS27	40°44'49.7"N 30°03'58.8"E	Forest, Kartepe	OP279183	OP279554	OP279530
Dorcadionini	*Dorcadion (Maculatodorcadion) triste* Frivaldszky, 1845	LDDT20	40°44'49.7"N 30°03'58.8"E	Forest, Kartepe	OP279181	OP279552	OP279532
Mesosini	*Mesosa* (*Aplocnemia*) *obscuricornis* Pic, 1894	LMMO32	40°41'57.5"N 29°54'28.6"E	Başiskele Countryside	OP279180	OP279544	OP279514
Mesosini	*Mesosa* (*Aplocnemia*) *obscuricornis* Pic, 1894	LMMO362	40°41'04.0"N 29°53'42.0"E	Başiskele Countryside	OP279179	OP279545	OP279513
Pogonocherini	*Pogonocherus* (*Pogonocherus*) *perroudi* Mulsant, 1839	LPPP14	40°49'45.1"N 29°29'50.9"E	Forest, Gebze	OP279156	OP279568	OP279517
Pogonocherini	*Pogonocherus* (*Pogonocherus*) *perroudi* Mulsant, 1839	LPPP53	40°40'23.1"N 30°04'08.1"E	Forest, Kartepe	OP279158	OP279566	OP279516
Pogonocherini	*Pogonocherus* (*Pogonocherus*) *perroudi* Mulsant, 1839	LPPP359	40°49'15.6"N 30°02'22.9"E	Forest, Kartepe	OP279159	OP279567	OP279515
Pogonocherini	*Pogonocherus* (*Pogonocherus*) *perroudi* Mulsant, 1839	LPPP360	40°57'19.0"N 29°38'54.4"E	Forest, Gebze	OP279157	OP279565	OP279518
Pogonocherini	*Exocentrus* (*Exocentrus*) *lusitanus* (Linnaeus, 1767)	LAEL366	40°42'52.6"N 30°03'21.1"E	Timber Yard, Gebze	OP279145	OP279563	OP279486
Pogonocherini	*Exocentrus* (*Exocentrus*) *lusitanus* (Linnaeus, 1767)	LAEL29	41°00'26.6"N 29°55'15.4"E	Forest, Gebze	OP279146	OP279564	OP279487
Saperdini	*Saperda* (*Lopezcolonia*) *octopunctata* (Scopoli, 1772)	LSSO344	40°59'31.4"N 29°33'23.4"E	Forest, Gebze	-	-	OP279497
Saperdini	*Saperda* (*Lopezcolonia*) *octopunctata* (Scopoli, 1772)	LSSO39	40°49'04.7"N 29°29'54.2"E	Timber Yard, Gebze	OP279171	OP279578	OP279498

Table 1. (continues) Binomial names, voucher codes, sampling coordinates, localities and GenBank accession numbers of the specimens sampled from the East of Marmara Basin, Turkey

Tribe	**Species**	**Voucher ID**	**Coordinate**	**Locality**	**COI**	**16S rRNA**	**28S rRNA**
Saperdini	*Phytoecia (Helladia) praetextata* (Steven, 1817)	LPPP367	41°07'44.9"N 30°11'57.6"E	Kandıra Forest	-	OP279581	OP279500
Saperdini	*Phytoecia* (*Phytoecia*) *pustulata* (Schrank, 1776)	LPPP365	41°07'45.9"N 30°12'50.1"E	Kandıra Forest	OP279177	OP279580	OP279499
Saperdini	*Phytoecia* (*Phytoecia*) *pubescens* Pic, 1895	LPPP28	40°41'20.7"N 29°54'05.7"E	Başiskele Countryside	OP279178	OP279579	OP279501
Batocerini	*Batocera rufomaculata* (DeGeer, 1775)	LBBR369	n/a	Diyarbakır	OP279147	OP279538	OP279524
Phrynetini	*Phryneta leprosa* (Fabricius, 1775)	LPPL368	n/a	Cameroon (Intercepted in Port, Derince)	OP279135	OP279535	OP279488

Two datasets were prepared for the phylogenetic analysis. The first one was a global mitochondrial *COI* sequence dataset of the subfamily Lamiinae, which was configured by combining the sequences obtained in this study and retrieved from the BOLD taxonomy archive. To increase the reliability of the dataset, the sequences shorter than 658 bp were discarded. A representative haplotype was selected for each available species from each zoogeographic region based on Löbl and Smetana (2010) (i.e., Neotropical, Nearctic, Afrotropical, Oriental Australian, Palearctic: Europe, Asia, North Africa) (Supplementary Table S1).

The second dataset consisted of two mitochondrial and one nuclear marker sequences; all were produced in this study (Table 1). 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; Stamatakis, 2014), the basis of AICc, by greedy search.

Nucleotide Diversity, Phylogenetic Analysis

Diversity analyses were performed in DnaSP v5 (Librado & Rozas, 2009). Haplotype analysis for mitochondrial gene datasets; parsimony-informative mutations (Pin), the total number of mutations (Eta), nucleotide diversity (Pi), insertions and deletions (InDel), and guanine+cytosine (G+C) percentages were calculated for each gene region. The alleles of the nuclear *28S rRNA* gene region were determined. The p-distances were calculated in MEGA-X (Kumar et al., 2018). Outgroups were selected from closest relatives according to Haddad et al. (2018): *Spondylis buprestoides* (Linnaeus, 1758) (Spondylidinae: Spondylidini), *Arhopalus rusticus* (Linnaeus, 1758) (Spondylidinae: Asemini).

Phylogenetic trees were obtained using Maximum Likelihood (ML), and Neighbor-Joining (NJ) approaches. ML analysis was conducted by using the PhyML (Guindon et al., 2010) plug-in of Geneious Prime v2021 for spliced dataset (Table 1) of three gene regions (2257 bp) with 34 terminal, produced in this study; and a global dataset of the mitochondrial *COI* gene region, including the sequences produced in this study (Table 1) and retrieved from databases (658 bp) with a total of 441 terminal (Supplementary Table 1). For both datasets, ML analyses were carried out by 10,000 bootstrap pseudo-replicates with estimated parameters and the Tamura-Nei (TN93) substitution model for unpartitioned data. NJ analysis was conducted by tree builder in Geneious Prime v2021 with 10,000 bootstrap pseudo-replicates and TN93 substitution model for only the global *COI* dataset (Supplementary Table 1).

Divergence Time Estimation

The time-scaled Bayesian analysis was conducted by BEAST v1.10.4 (Drummond & Rambaut, 2007) under the Yule speciation process (Gernhard, 2008; Yule, 1925) and uncorrelated relaxed clock with lognormal distribution (Drummond et al., 2006). Jukes-Cantor (JC) substitution model was used for unpartitioned data after trial runs to determine the best fitting model and priors. Two Bayesian analyses were run for 100 million MCMC generations, sampled every 1000 steps, of which 10% were discarded as burn-in. The outputs merged by LogCombiner v1.10.4 and the maximum clade credibility (MCC) trees resulting from each run were interpreted by TreeAnnotator v1.10.4 in the BEAST package. Tracer 1.7 (Rambaut et al., 2018) was used to assess if the implemented parameters provide effective sample sizes (ESSs). The root age was calibrated to 147.03 mya, as the most common recent ancestor (MRCA) of Lamiinae and Spondylidinae, according to Nie et al. (2021). The MCC trees were annotated in FigTree v1.4.4

(Rambaut, 2014), and the visual adjustments were performed in CorelDraw v.1512 (corel.com).

RESULTS

Genetic Diversity and Phylogenetic Analysis

A summary of the diversity statistics and maximum p-distances of each sequence set is presented in Table 2. The hypothetical phylograms resulting from the NJ and ML analyses of the global dataset of *COI* barcode region adumbrated relationships, particularly at the generic level. Each sequence obtained in this study from the East of Marmara Basin was clustered with the European conspecifics, congeneric or relatives at the *COI*-based NJ and ML trees (Supplementary Fig. S1, Supplementary Fig. S2).

Table 2. Characteristics of the nuclear and the mitochondrial genes produced in this study

Gene Region	**N**	**h/a**	**bp**	**Hd**	**SDHd**	**Pi, π**	**G+C (%)**	**InDel**	**S**	**Eta**	**Pin**	**Dmean (%)**	**Dmax (%)**
mtCOI	49	34	658	0,983	0,008	0,16837	32.9	n/a	319	500	270	16.84	23.10
mt16S rRNA	47	25	514	0,958	0,013	0,16550	26.1	41	230	350	217	16.55	26.25
n28S rRNA	47	24	1050	n/a	n/a	0,05446	59.9	42	212	s265	n/a	5.56	8.54

N number of analysed sequences; n number of species; h/a haplotype number for mitochondrial sequences, allele number for nuclear sequences, bp base pair, Hd haplotype diversity, SDHd standard deviation of haplotype diversity, Pi, π nucleotide diversity, G+C (%) guanine and cytosine bias, InDel number of total Indel events, S segregating sites, Eta total number of mutations, Pin parsimony informative sites, D Mean p-distance, Dmax Maximum p-distance between specimens.

The phylogram hypothesised by the ML approach of spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (Table 1) of Lamiinae schemed a pattern as follows:

The first main branch including [(Lamiini+Dorcadionini) + (Monochamiini+Batocerini)] + Mesosini, the second Phrynetini+Pogonocherini (including Exocentrini), the third

Agapanthiini, fourth Acanthocinini+Acanthoderini, and the last one Saperdini+Phytoeciini. For traceability, we mostly used generic names of the representatives of tribes in the following text.

The phylogenetic tree resulting from ML analysis of the spliced dataset of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions retrieved the Lamiini+Dorcadionini branch and the Monochamiini+Batocerini branch as sisters with 81% bootstrap. It stated Mesosini at the base of this group (Fig. 1). The *COI*-based NJ and ML gene trees supported the affiliation of the genera *Morimus* (Lamiini) and *Dorcadion* (Dorcadionini), besides the genus *Iberodorcadion* (Dorcadionini) nested in the same group, sister to *Morimus*+*Dorcadion* branch. The other genera splinted from the same node in NJ tree were *Paraleprodera* and *Lamia* (Lamiini), *Imantocera* (Gnomini), *Batocera* and *Apriona* (Batocerini). (Supplementary Fig. S1 and Supplementary Fig. S2). *Peblephaeus* (Lamiini) was stated at the basal-most of the group at the NJ tree (Supplementary Fig. S1). *Mesosa obscuricornis* was clustered with congenerics and *Synaphaeta* (Mesosini) and *Acalolepta* (Lamiini) (Supplementary Fig. S1 and Supplementary Fig. S2). The Phrynetini+Pogonocherini branch was recovered as the sister group of Lamiini and the relatives mentioned above (Fig. 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 (Fig. 1). 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 the basal-most of the *COI*-based ML tree.

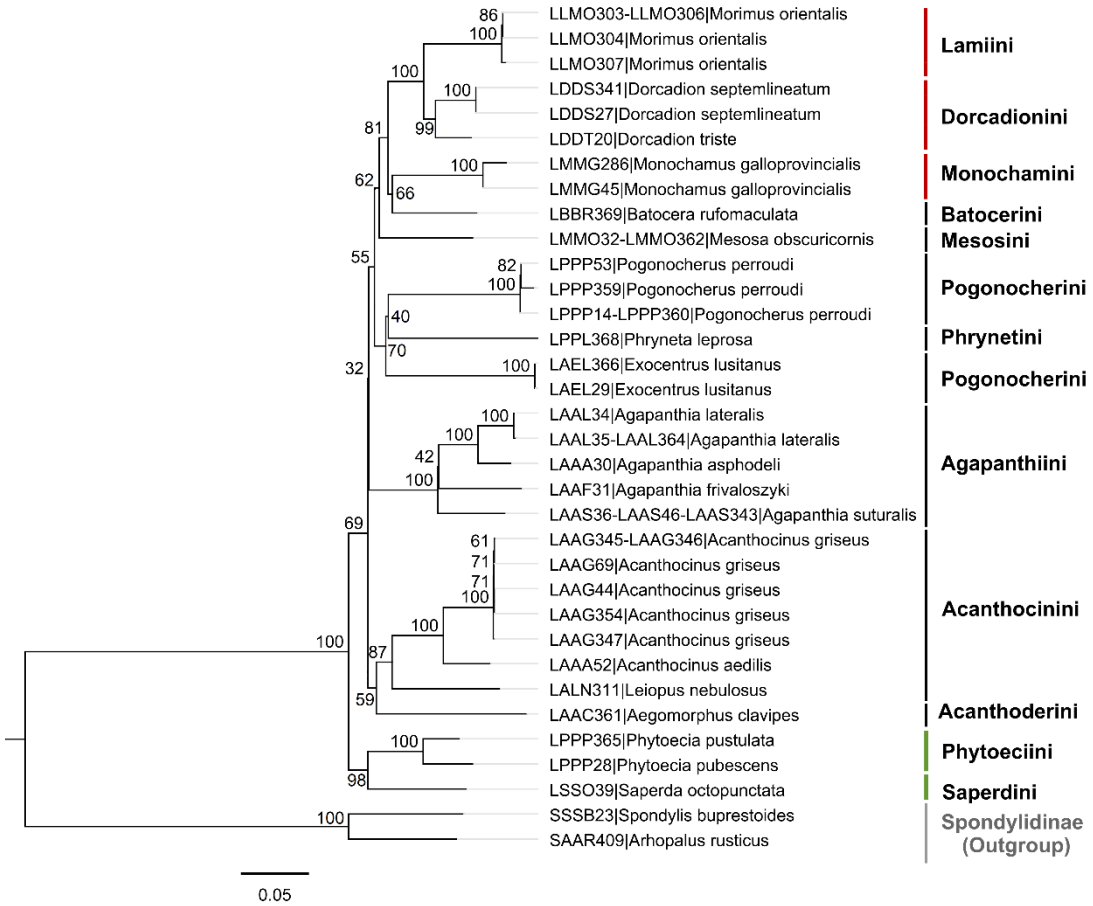

Figure 1. Phylogenetic relationships recovered by maximum likelihood (ML) analysis of spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions of Lamiinae species sampled from the East of Marmara Basin, Turkey. The scale bar represents substitution per site; bootstrap supports are indicated beside nodes.

The two genera in Pogonocherini, *Ecyrus* and *Exocentrus*, were clustered with *Rosalba sp. (Apomecynini)* and *Bactriola sp.* (Forsteriini) at the *COI*-based ML tree Supplementary Fig. S2). The genus *Phryneta* was clustered with the members of the genera *Anaesthetis and Pseudanaesthetis* from the tribe Desmiphorini (Supplementary Fig. S2), while *Exocentrus* was clustered with *Desmiphora* (Desmiphorini) at the *COI*-based NJ tree (Supplementary Fig. S1).

The tribe Agapanthiini was represented by the genus *Agapantia* at the ML tree of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions,

stated at the base of the groups mentioned above with trivial statistical support (Fig. 1). At the *COI*-based NJ and *COI*-based ML trees, two other species from Agapanthiini, *Calamobius filum* and *Pothyne virginalis* were clustered in the *Agapantia* genus group (Supplementary Fig. S1, Supplementary Fig. S2).

The sole representative of the Acanthoderini tribe *Aegomorphus clavipes,* stated at the 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 (Fig. 1). At the *COI*-based NJ and *COI*-based ML trees tree *Aegomorphus modestus* was joined to *A. clavipes*. They were clustered with some other Acanthoderini members such as genera *Acanthoderes, Paradiscopus Psapharochrus* and *Steirastoma* (Supplementary Fig. S1, Supplementary Fig. S2).

The basal-most branch of the ML tree of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions included Saperdini and Phytoeciini (Fig.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 *Stenostola* from the tribe Saperdini. In the genus group of *Phytoecia*, the genus *Oberea* (Obereini) was nested. These two groups were stated as sister clades (Supplementary Fig. S1, Supplementary Fig. S2).

Divergence Time Estimation

The time-scaled Bayesian analysis of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (Table 1) suggested a mostly congruent topology with the ML analysis (Fig.1, Fig. 2). The MRCA of Lamiinae rely on the taxa involved in the analysis, raised around the Middle and Late Cretaceous, 89.55 mya (95%

highest posterior density (HPD) interval 113-73 mya). The common ancestor of the Lamiini and its close relatives (Dorcadionini, Monochamiini, Batocerini and Mesosini appeared 57.43 (95% HPD: 74-44) mya around the Late Cretaceous and Eocene epoch of the Paleogene period. Also, appearing times of the earliest common ancestors of the other groups were from Late Cretaceous to the different epochs of Paleogene. Phrynetini+Pogonocherini (including Exocentrini) and Acanthocinini+Acanthoderini emerged in Late Cretaceous to Eocene 53.91 (HPD: 72-38) mya and 65.29 (95% HPD: 87-45) mya, respectively. The MRCA of Saperdini+Phytoeciini emerged in 53,21 (95% HPD: 81-31) mya, somewhere from Late Cretaceous to Oligocene. While the emerging time of the MRCA of the Agapanthiini genus group was 40.86 (95% HPD: 61-28) mya, the emerging time of the MRCA of the tribes Dorcadionini, Lamiini and Monochamini was 39.52 (95% HPD: 53-27) mya. Besides, the MRCA of these three tribes was younger than all MRCAs of the other tribes (Fig. 2). The intrageneric diversification relies on the taxa involved in the analysis that occurred during Cenozoic. The members of *Agapanthia* arose during Paleogene 48.86 (HDP: 59-28) mya, earlier than *Phytoecia* members during Neogene 19.38 (30-9) mya. The last speciation event within our sampling corresponded to the Quaternary and occurred within the genera *Dorcadion* and *Acanthocinus*.14.98 (95% HPD: 24-8) and 1.08 (HDP:1.1-1.5) mya, respectively.

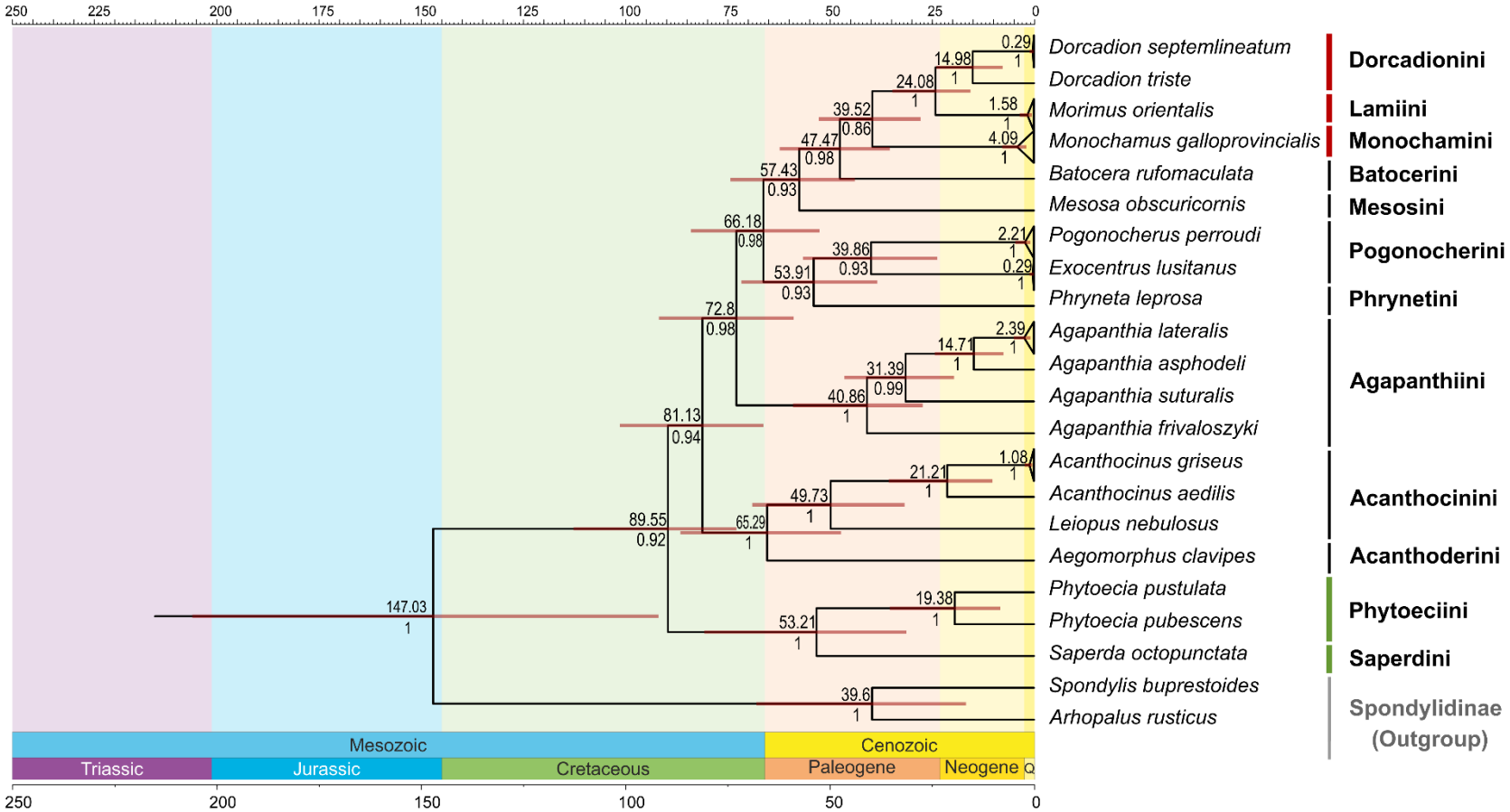

Figure 2. Divergence times recovered by Bayesian analysis of spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions of Lamiinae species sampled from the East of Marmara Basin, Turkey. The brown bars correspond to the 95% highest posterior density (HPD) interval; the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

DISCUSSION

The estimations of previous studies on the emergence of the cerambycids have resulted in different dates. The earlier studies have pointed out somewhere from the Late Cretaceous (Gómez-Zurita et al., 2007) to the Early Cretaceous (Wang et al., 2013; Zhang et al., 2018), while recent studies, which were conducted with broader samplings and genetic data, have pointed out earlier emergence time, around late Jurassic to early Cretaceous (Ashman et al., 2022; Nie et al., 2021). On the other hand, the studies for resolving phylogenetic relationships within the subfamily Lamiinae are mostly consistent with each other and with our findings.

Consistency between the topologies suggested by the ML and the time-scaled BI analyses of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions about the affinity of the genera *Morimus* (Phrissomini syn. of Lamiini) and *Dorcadion* (Dorcadionini) (Fig. 1, Fig. S2) supports the findings of Giannoulis et al. (2020), who concluded from the karyotype data and the partial *COI* gene sequences. The absence of *Herophila* (Phrissomini syn. of Lamiini) in our datasets, unlike Giannoulis et al. (2020)'s, prevented us from testing whether this species was closer to *Morimus* than *Dorcadion* or *Lamia* (Lamiini), as they claimed. Also, dissimilar to Giannoulis et al. 2020, our results do not support the placement of *Phytoecia* (Phytoeciini) at the base of the Lamiini+Dorcadionini branch together with *Monochamus* (Monochamini), but support the station of *Monochamus* and *Batocera* closely, conformable with the results of de Ashman et al. (2022), and de Santana Souza et al. (2020). Before the studies mentioned above, the affinity of the genera *Monochamus* to the tribe Lamiini had been shown by Ohbayashi et al. (2009), who investigated the relationships of *Anoplophora* from the tribe Lamiini with samples collected from Japan based on the mitochondrial *COI* gene region.

They showed the clustering of the genera *Anoplophora* and *Monochamus* in the same lineage. Also, Gorring (2019) suggested that Monochamini is the synonym of Lamiini. de Santana Souza et al. (2020) underlined that Dorcadionini, Gnomini, Monochamini and Rhodopinini should be considered synonyms of Lamiini. Our *COI*-based NJ and ML trees supported the affiliations of *Morimus* (Lamiini), *Dorcadion* and *Iberodorcadion* (Dorcadionini), *Imantocera* (Gnomini), *Paraleprodera* (Lamiini) and *Lamia* (Lamiini), *Batocera* and *Apriona* (Batocerini) (Supplementary Fig. S1, Supplementary Fig. S2). Also, *Morimus*, *Dorcadion*, *Monochamus*, and *Batocera* were clustered in our ML and time scaled-BI analyses of the spliced datasets of mitochondrial *COI* and *16S rRNA* and nuclear *28S rRNA* gene regions (Fig. 1, Fig. 2). Ren et al. (2021) is another recent study, supported the sisterships of Batocerini and Lamiini. The MRCA of the group, which included *Monochamus* and *Batocera*, appeared around 63 mya at the chronogram of Ashman et al. (2022), 54.9 mya of Nie et al. (2021), and 47.47 (95% HPD: 63-36) mya at our chronogram, Eocene epoch of Paleogene. This emergence date is closer to the present than the ages of MRCAs of other tribes (Fig. 2). Contemplating the results of the present study and the previous studies mentioned above, Dorcadionini, Gnomini, Monochamini (in terms of *Monochamus*) should be revised. Also, we support the expectations of Lacordaire (1869) and Pascoe (1866) on the close relation of Batocerini and Monochamini.

The proximity of Mesosini to the cluster of Lamiini had been shown in the phylogenetic trees of de Santana Souza et al. (2020) and supported by our findings. (Fig. 1, Fig. 2, Supplementary Fig. S1 Supplementary Fig. S2). However, unlike de Santana Souza et al. (2020), in our phylogenetic trees, *Mesosa* was not in the same sub-cluster as *Saperda* (Saperdini). The age of MRCA of the groups in which *Mesosa* and *Batocera* were

included was 123.45 mya at the chronogram of Nie et al. (2021) and 57.43 mya (95% HPD: 74-44 mya) at our chronogram, which corresponds to early Eocene.

Phrynetini, represented by *Phryneta leprosa,* was nested in Pogonocheriini, represented by *Pogonocherus* and *Exocentrus,* in our phylogenetic trees, while it was at the base of the Acanthoderini, according to de Santana Souza et al. (2020)'s results. 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 Fig. 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 these tribes by extensive sampling and broader genetic data. The MRCA of *Pogonocherus* and *Exocentrus* emerged around 65 mya at the edge of the Cretaceous and Paleogene, according to the chronogram of Ashman et al. (2022), while it is 39.86 (95% HPD: 56-26 mya) around Mid-Paleogene, according to the hypothesised chronogram in the present study (Fig. 2).

Aegomorphus clavipes (Schrank, 1781), the sole representative of the Acanthoderini, was stated at the base of Acanthocinini in our time scaled-BI and ML analyses, while it was clustered with *Phryneta leprosa* from Phrynetini in de Santana Souza et al. (2020). According to the chronogram of Ashman et al. (2022), MRCA of the subfamily Lamiinae emerged at around 105 mya; *Acanthocinus griseus* (Acanthocinini) birth by MRCA of the Lamiinae, and stated at the base of the Lamiinae clade. According to our chronogram, the emergence of the MRCA of the crown group was 89.55 (95% HPD:113-73) mya. Besides, the MRCA of Acanthocinini and Acanthoderini was around 65.29 (95% HPD: 87-45) million years old and emerged at the end of the Cretaceous.

In de Santana Souza (2020), Agapanthiini was only monophyletic in BI, not ML tree; *Calamobius filum* was clustered with the genus *Agapanthia,* while *Hippopsis* sp. from this tribe was clustered out of this group. Similar to de Santana Souza et al. (2020), at the COI-based NJ and ML gene trees, *Hippopsis* sp. was clustered out of the Agapanthiini group, with *Omosarotes singularis* from the tribe Acanthomerosternoplini (Supplementary Fig. S1). Considering our chronogram, the MRCA of four species of *Agaphantia* appeared 40.86 (95% HPD: 61-28) in Paleogene (Fig. 2), while the MRCA of two *Agaphantia daurica* individuals dated 51.79 mya in Nie at el. (2021).

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 Fig. S1, Supplementary Fig. S1). The close relationship between *Phytoecia* and *Oberea* was also supported by Ashman et al. (2022), and their MRCA was dated 36 mya. According to our chronogram, the MRCA of Saperdini and Phytoeciini was 53.21 (95% HPD: 80-34) million years old. Same as Ren et al. (2021), 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.

The intrageneric diversification relies on the taxa involved in the analysis that occurred during Cenozoic. The members of *Agapanthia* arose during Paleogene 48.86 (HDP: 59-28) mya, earlier than *Phytoecia* members during Neogene 19.38 (HDP:30-9) mya. The last speciation event within our sampling corresponded to the Quaternary and occurred

within the genera *Dorcadion* and *Acanthocinus*, 14.98 (95% HPD: 24-8) mya and 21.21 (HDP:31-10) mya, respectively. According to the chronogram of Ashman et al. (2022), intrageneric diversification occurred during Neogene, similar to our results. According to the chronogram of Dascălu et al. (2022), the beginning of speciation within the genera *Dorcadion* was around 9.8 mya. Considering all recent studies and the present study, the emergence time of the subfamily Lamiinae is Jurassic to early Cretaceous, and the current species primarily emerged during the Neogene period.

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Conflict of interest

The authors report that there are no competing interests to declare.

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