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Beetle bioluminescence outshines extant aerial predators

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We understand very little about the timing and origins of bioluminescence, particularly as a predator avoidance strategy. Understanding the timing of its origins, however, can help elucidate the evolution of this ecologically important signal. Using fireflies, a prevalent bioluminescent group where bioluminescence primarily functions as aposematic and sexual signals, we explore the origins of this signal in the context of their potential predators. Divergence time estimations were performed using genomic-scale datasets providing a robust estimate for the origin of firefly bioluminescence as both a terrestrial and as an aerial signal. Our results recover the origin of terrestrial beetle bioluminescence at 141.17 (122.63–161.17) Ma and firefly aerial bioluminescence at 133.18 (117.86–152.47) Ma using a large dataset focused on Lampyridae; and terrestrial bioluminescence at 148.03 (130.12–166.80) Ma, with the age of aerial bioluminescence at 104.97 (99.00–120.90) Ma using a complementary Elateroidea dataset. These ages pre-date the origins of all known extant aerial predators (i.e. bats and birds) and support much older terrestrial predators (assassin bugs, frogs, ground beetles, lizards, snakes, hunting spiders and harvestmen) as the drivers of terrestrial bioluminescence in beetles. These ages also support the hypothesis that sexual signalling was probably the original function of this signal in aerial fireflies.

1. Introduction

Bioluminescence has evolved independently almost 100 times across both eukaryotes and prokaryotes (e.g. insects, crustaceans, other marine invertebrates, fish, protists, fungi and bacteria [1–4]). The function of bioluminescence varies across organisms including: prey attraction, [5–8] predator avoidance [9,10], counterillumination [11,12], sexual communication [13,14] and spore dispersal [15,16]. In the terrestrial environment, bioluminescence is principally used as an aposematic signal [10,17–19].

While Lampyridae (fireflies) are perhaps the most well-known group of bioluminescent beetles, there are four additional extant families that contain bioluminescent species in the superfamily Elateroidea: Elateridae, Sinopyrophoridae, Phengodidae and Rhagophthalmidae. Two independent origins of bioluminescence in Elateroidea were suggested by the phylogenetic analysis

of Martin *et al*. [20] and Kusy *et al*. [21]. One origin of bioluminescence in the common ancestor of fireflies and their relatives (Lampyridae, Rhagophtalmidae, Phengodidae and Sinopyrophoridae) was supported by Kusy *et al*. [21], and a second independent origin in click beetles (Elateridae). Evidence for these two origins is corroborated by Fallon *et al*. [22], who provided genomic evidence that strongly supports the independent evolution of elaterid and lampyrid luciferases. We focus our analysis on the origin of bioluminescence in the lampyroid group, including the families Lampyridae, Phengodidae and Rhagophthalmidae. All known lampyroid larvae are bioluminescent [23], suggesting larval bioluminescence is the ancestral state for each of these families and their common ancestor. These larvae are also largely terrestrial [13].

Current evidence suggests that larval elateroid bioluminescence probably originated as a predator avoidance strategy in fireflies [20,24–27] by allowing these ground-dwelling invertebrates to advertise their chemical defences to potential predators [17,18,26,27]. For example, Underwood *et al*. [28] found that firefly predators learned to avoid distasteful prey when it was associated with light, ultimately resulting in effective predator avoidance. This demonstrates the effectiveness of bioluminescence, when accompanied by chemical defences, as a strong aposematic signal.

Independent from larval bioluminescence, adult firefly bioluminescence (with light organs in different abdominal segments: [24]) subsequently evolved separately in several firefly lineages [20,24,29]. Today, this adult bioluminescence is mainly used for sexual communication in the form of complex aerial bioluminescent courtship displays by male fireflies, followed by a response from usually sedentary conspecific females on the ground or in the vegetation above [30]. There is evidence that adult firefly bioluminescence also accelerates avoidance learning by potential predators and thus also functions as an aposematic signal for aerial predators (i.e. bats [31,32]). However, it is unclear how adult firefly bioluminescence originated. In fact, despite the modern interactions between larval and adult fireflies with many different predator groups [33,34], and a few claims that specific predators caused adult firefly bioluminescence [32], none of these groups can yet be attributed to the origin of bioluminescence as an aposematic signal in fireflies.

To investigate the origins of bioluminescence in the predator context, we distinguish between terrestrial bioluminescence and aerial bioluminescence to test which predator groups may have driven the origin of larval bioluminescence in the common ancestor of Lampyridae (fireflies), Rhagophthalmidae (railroad worms) and Phengodidae (glowworms), and which predator groups may have contributed to the origin of adult bioluminescence in fireflies. From a predator perspective, adult larviform or wingless (apterous or brachypterous) females tend to be active on the ground and thus display terrestrial bioluminescence. The origin and evolution of terrestrial bioluminescence as an aposematic signal would probably have been driven by terrestrial predators (e.g. carabid beetles, arachnids, centipedes, amphibians, reptiles and rodents) [33]. By contrast, winged (pterous) adult males use aerial bioluminescence (above ground) in their complex and highly visible courtship displays, and their winged adult females also tend to respond with individual flashes from higher up in the vegetation (above ground or aerial bioluminescence). For aerial bioluminescence to arise as an aposematic signal, aerial predators (e.g. bats, dusk/night active birds [33,35]) would have to pre-date or coincide with the origin of aerial bioluminescence displays. Most importantly, to contribute to the origin of terrestrial and/or aerial (above ground) bioluminescence in fireflies, a potential predator group would have to pre-date or coincide with the respective origins of terrestrial or aerial bioluminescence.

Here, we estimate when terrestrial, larval beetle bioluminescence (Lampyridae, Phengodidae and Rhagophthalmidae) originated, as well as the time of the first origin of adult, aerial bioluminescence in fireflies (Lampyridae). We take advantage of eight described fossils and two genomic-scale phylogenies [36,37] to date the origins of bioluminescence in elateroid beetles. Both phylogenies were focused on elateroid beetles, but with a different emphasis in taxon sampling. Martin *et al*. [36] focused on subfamilies and tribes within Lampyridae while Douglas *et al*. [37] focused on subfamily relationships within the Elateridae. By dating each of these two topologies independently, we can take into account how taxon sampling and fossil placement may affect the resulting divergence time estimates, thereby providing the most robust estimate for the age of bioluminescence in this group to date. We use the resulting age estimates to examine the presence of potential terrestrial and aerial predator groups at the estimated origins of terrestrial bioluminescence in beetles and aerial bioluminescence in fireflies to test hypotheses on the roles of these predators in the origin of bioluminescence. These results shed light on potential selective agents for the origin of beetle bioluminescence, both on the ground and in the air, ultimately giving rise to some of the most diverse and captivating light displays seen in the terrestrial environment.

2. Material and methods

(a) Phylogenies used for divergence time estimation

Bayesian divergence time estimation combines phylogenetic hypotheses with prior knowledge of molecular clocks or, in this case, the fossil record allowing for investigations into the time of origin for organisms and their ecological innovations [38,39]. Fossil calibrations are known to have a significant impact on divergence time estimation depending on their placement and the ages provided by the user [40,41]. We obtained both the tree file and alignments from Martin *et al*. [40] and the alignments provided by Douglas *et al*. [37] to perform divergence time estimation. The alignments from Douglas *et al*. [37] were run in ModelFinder [42] and then used to reconstruct a topology in IQ-Tree v. 1.6.12 [43] using ultrafast bootstraps [44] and compared with the published phylogeny to confirm congruence.

We used *chronoPL* as implemented in the R package [45] APE [46] to produce fixed topologies of the Martin and Douglas trees for divergence time analyses [47,48]. Using a fixed topology cuts down on the necessary computing time and ensures that the resulting ages are not impacted by minor differences in topology. This program allowed us to transform the topologies into ultrametric trees and adjust the node heights such that they were within the bounds of the fossil prior distributions we provided in subsequent divergence time estimation (see 'Fossil selection and placement' below).

(b) Fossil selection and placement

We selected a total of nine fossils with a focus on those that could provide reliable calibration points for major clades across each topology. Two of these fossils were used on both topologies.

The placement of the fossils was determined based on morphological similarities with extant taxa and currently proposed placements (see electronic supplementary material, table S1). Other described fossils were not included (e.g. [49,50]) when older fossil representatives in their corresponding clades were available. Relative ages for each fossil were obtained by the Paleobiology Database [51], which uses stratigraphic information to assign ages to geologic deposits.

For the Martin *et al*. [36] topology, four fossil priors were placed based on the published classification and study of the original descriptions and images. One was placed at the base of Luciolinae (*Protoluciola albertalleni* [52]) and one at the node Phengodidae + Rhagophthalmidae (*Cretophengodes azari* [53]), a third fossil was placed at the base of the subfamily Lamprohizinae (*Phausis fossilis* [54,55]) and the fourth at the base of the *Photinus* (*Photinus kazantsevi* [56]). Due the questionable assignment of *P. kazantsevi* to the genus *Photinus* and its therefore problematic placement within the phylogeny (M.A.B. & L.F.L.D.S. 2022, unpublished data), we also ran a subset of analyses on the Martin topology excluding this fossil. Removal of the *Phontinus* fossil prior had minimal impact on the ages for surrounding nodes (−1 to +8 Ma). For the Douglas *et al*. [37] topology, we used two fossil priors that overlapped with the calibration points used for Martin *et al*. [36]. These were placed at the base of Lampyridae (*Protoluciola albertalleni* [52]) due to a lack of species determinations used by Douglas *et al*. [37]. The other was once again placed at the base of Phengodidae + Rhagophthalmidae (*Cretophengodes azari* [53]). Additionally, we placed four fossils to constrain major Elateridae clades across the topology: one at the base of the Agrypninae (*Ageratus delicatus* [57]), one at the base of the Lissomini (*Lissomus taxodii* [57]), one at the base of the Negastrini (*Ganestrius elongatus* [57]) and one at the base of *Cardiophorus* (*Cardiophorus exhumatus* [57]). Each of these four fossils was assigned a soft maximum of 242 Ma based on the oldest Elateridae (*Elateridium* spp. [57]).

When using fossil specimens as calibration points, we used an exponential probability distribution as it allows relative ages of each fossil to be used as 'hard' minimums, as there is no need to sample ages younger than the fossil evidence being used. By contrast, maximum ages are 'soft' allowing the divergence time estimation to sample ages older than that proposed maximum, but do so by decreasing the probability of those ages as they get further from that maximum age [58]. Furthermore, an exponential distribution is preferred in the absence of additional information as it requires only two parameters (minimum age and the mean) to be set by the user over the three required for a log-normal distribution [58]. We generated potential age distributions by setting the relative age of the fossil as the hard minimum, and adjusting the mean age such that the 95% quantile of the exponential distribution matches that of our soft maximum, but does not exceed the age of the oldest known representative of the parent clade (i.e. the maximum age for the subfamily Agrypninae does not exceed the age of the oldest known representative for the family Elateridae).

(c) Divergence time estimation

Next, we employed BEAUTi [59] to set all analysis parameters such as fossil placement and age distributions, fixed starting trees, and the tree and clock models. To account for sensitivity to model choice, four different analyses were performed on each dataset (i.e. Martin and Douglas). Each analysis used a different combination of tree and clock models which included: birth–death (BD) and relaxed clock exponential (RCE), BD and relaxed clock log normal (RCLN), Yule and RCE, Yule and RCLN. Analyses were performed. Divergence time estimation, using the files prepared in BEAUTi, were run in BEAST v. 2.6.0 [59] via the CIPRES Science Gateway v. 3.3 (www.phylo.org). To ensure convergence of our Bayesian analyses, and to determine burn-in, the resulting log file for each analysis was viewed in Tracer v. 1.7.1 [60]. Analyses were run until effective sample sizes of parameters of interest were greater than 100, with many parameters being greater than 200 (this required a chain length of 200 000 000 and 450 000 000 for Martin and Douglas, respectively). Lastly, TreeAnnotator v. 1.10.4 [38] was used to generate consensus ages for each analysis after a burn-in (10–50%) was discarded. All ages are reported as the median age followed by the 95% highest posterior density interval (HPD).

(d) Ancestral state reconstruction

We evaluated the origin of bioluminescence for both the larval and adult life stages. Larval bioluminescence was coded as absent (0) or present (1). In these analyses, larval bioluminescence was considered equivalent to adult terrestrial bioluminescence because all known larval fireflies lack wings and thus would not be expected to be displaying in the aerial environment during this life stage [13]. Adult bioluminescence was coded as either absent (0), female-only terrestrial bioluminescence, with non-bioluminescent males (1), or aerial bioluminescence of either males and females (2) (electronic supplementary material, table S2). Maximum parsimony and maximum-likelihood ancestral state reconstructions were conducted for both datasets with Mesquite v. 3.61 [61].

(e) Potential predators

If larval beetle bioluminescence arose to advertise a chemical defence in terrestrial larvae then there must have been predation pressure by terrestrial predators selecting for this trait. If adult firefly bioluminescence arose as an aposematic signal during aerial displays then there must have been aerial predators preying upon these aerial bioluminescent individuals. Terrestrial and aerial animal groups known to prey on fireflies (e.g. insectivores) were compiled from [33,62], and authors' personal observations (see results). Extinct insectivorous groups (e.g. Pterosauria) were also considered [63]. To assess the potential role of each predatory group in the origins of terrestrial (larval and flightless females) and aerial adult beetle bioluminescence, predator group ages were compared with our estimates for the origin of terrestrial and aerial bioluminescence. The ages of each firefly predator group were gathered from the literature [41,63–71] (electronic supplementary material, table S3).

3. Results and discussion

Terrestrial (larval) bioluminescence is an ancestral trait in Lampyridae (and their relatives: Phengodidae and Rhagophthalmidae), preceding the origin of adult aerial bioluminescence (figure 1) [24] (see also electronic supplementary material, figures S1–S4), this topological result is supported by 100% bootstraps as reported by Martin *et al.* [36] (figure 1). Our results recover the origin of terrestrial beetle bioluminescence at 141.17 (122.63–161.17) Ma. This was followed by the origin of aerial bioluminescence, recovered to originate at a more derived node in fireflies (Lampyridae), at 133.18 (117.86–152.47) Ma (figure 1). These ages are corroborated by our analysis of the Douglas *et al.* [37] dataset. These independent divergence time estimates recovered the origins of terrestrial bioluminescence at 148.03 (130.12–166.80) and aerial bioluminescence at 104.97 (99.00–120.90). Both sets of recovered ages are older than previous

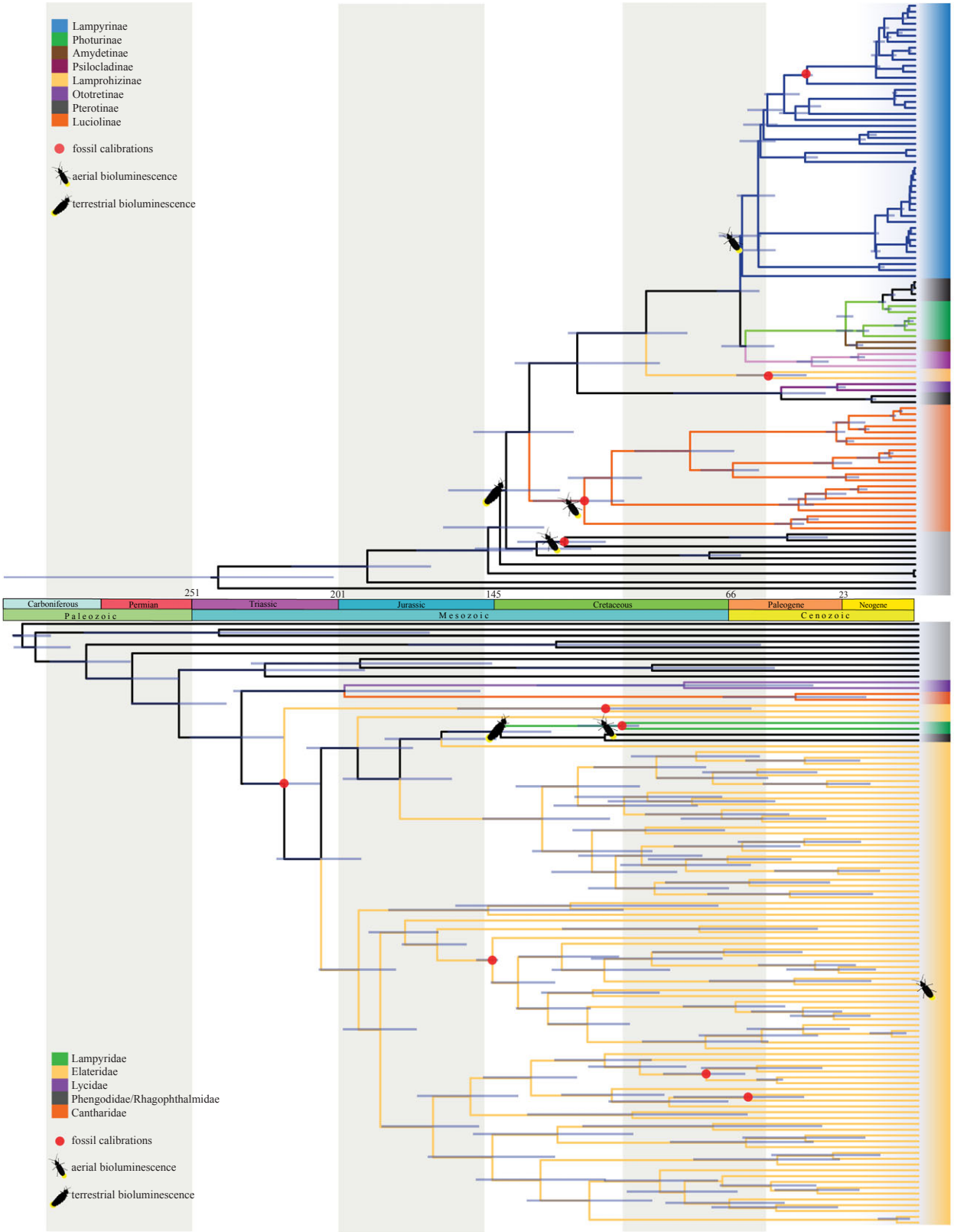

Figure 1. Two divergence-time calibrated phylogenies of Elateroidea using LN and BD (based on data from Martin *et al*. [36], top, and Douglas *et al*. [37], bottom) with results of ancestral state reconstruction for both terrestrial and aerial bioluminescence depicted at appropriate nodes. Grey vertical bars represent 50 million years. (Online version in colour.)

estimations and preclude modern aerial predators as selective agents for the origin of beetle bioluminescence.

(a) Divergence time estimation

Accurate divergence time estimation relies on a combination of broad taxon sampling, an accurate phylogenetic reconstruction, and sufficient breadth and depth of fossil sampling [40,41,72]. A limited fossil record, the difficulty of placing extinct taxa, and the immense extant diversity for a group like Elateroidea (21 000 described species), have led to continued disagreement for the ages of these groups. In addition, the systematics of the group has a level of uncertainty (see [21,37]). This broader-scale uncertainty, however,

will have little impact on the ages recovered for the bioluminescent taxa here. Previous estimates for the origin of Elateroidea ranged from 220 to 130 Ma, and estimates for the origin of Lampyridae ranged from 130 to 75 Ma [73–75]. These large ranges were probably due to a combination of limited taxon sampling for lampyrids and limited fossil calibration points. For example, Bocak *et al*. [74] hypothesized the age of Elateroidea to be approximately 220 Ma and Lampyridae as approximately 130 Ma based on two extant fireflies, 19 total Elateroidea taxa, and a single elateriform fossil calibration point (15 gene dataset). Similarly, McKenna *et al*. [73] estimated the age of Elateroidea to be approximately 130 Ma and Lampyridae to be approximately 78 Ma, with a dataset of four firefly taxa (eight gene dataset). In a subsequent analysis, McKenna *et al*. [73] reconstructed a phylogeny of Coleoptera and estimated the of Elateroidea to be approximately 187 Ma and approximately 120 Ma for the branch leading to Lampyridae; however, this study included only a single lampyrid species and no internal elateroid fossil calibration, instead using an ancestral click beetle at the base of Elateroidea + Byrroidea (4800 gene dataset). Oba *et al*. [25] reconstructed the luciferase gene for the common ancestor of elateroids and estimated the origin for this luciferase at approximately 102.55 Ma using seven taxa and a single gene tree (18S).

Our analyses based on Martin *et al*. [36] with 436 loci, 88 firefly species (98 taxa total) and four fossil calibrations across the topology recovered the age of terrestrial bioluminescence as 141.17 (122.63–161.17) Ma and aerial bioluminescence of 133.18 (117.86–152.47) Ma. While the divergence time estimates we performed include largely overlapping HPD intervals, limiting the discussion about the amount of time between the origins of terrestrial and aerial bioluminescence, the order of these origins is strongly supported and corroborated by previous studies [24,36,37]. Our analyses based on Douglas *et al*. [37] with 958 loci, two firefly species (an additional two 'lampyroids') (88 taxa total) and seven fossil calibrations. Divergence time estimates for this dataset recovered the root age for Elateroidea as 278.61 (262.03–294.86) Ma, the age of terrestrial bioluminescence as 148.03 (130.12–166.80) Ma, and the age of aerial bioluminescence at 104.97 (99.00–120.90) Ma. It also recovered an independent origin of adult bioluminescence in Elateridae where Pyrophorini branches off at 115.42 (91.70–133.70) Ma.

These divergence time estimates for Lampyridae are older than previously published estimates, probably due to improved taxon sampling. McKenna *et al*. [73,76] had limited lampyrid and broader elateroid taxon sampling with limited fossil calibrations that resulted in much younger ages for Lampyridae, and Coleoptera overall, which was already noted by others [41,77]. Our age estimates are closely aligned to the estimates of Bocak *et al*. [74], which is probably due to their larger elateroid sampling and ingroup fossil calibrations (e.g. *Elaterophanes*). Although not included in this study, the placement of the elaterid-like clicking beetles, Sinopyrophoridae, was recovered by Kusy *et al*. [21] as sister to the Lampyridae + Phengodidae + Rhagophthalmidae. If this placement was confirmed, it would only further increase the age of the origin of bioluminescence in this group. With updated clade ages, and the species-level biological information gathered for bioluminescence across our phylogeny, we can now examine the validity of previous hypotheses relating to predation and the origin of firefly bioluminescence.

(b) Age of bioluminescence in beetles

Clade ages for bioluminescence were largely congruent between all different clock and tree models implemented with minor variations in estimated ages reported between each parameter combination (electronic supplementary material, table S4). The Yule model does not account for any extinction and elateroid beetles have experienced at least two global extinction events. Thus, we have focused our discussion going forward on the ages resulting from BD tree models and those using the Martin *et al*. [36] dataset as the taxon sampling for bioluminescent taxa is much greater. However, we report the variation in age for each model to demonstrate the robustness of our analyses. The age estimates for each clock model combination largely overlapped for the BD models; here we discuss the RCLN model, which assumes the branch rates are normally distributed and has been shown to more precisely estimate ages and therefore is widely used [78,79]. Our ancestral state reconstruction placed the origin of terrestrial bioluminescence operating as an aposematic signal during the early Cretaceous period. This was followed by the origin of aerial bioluminescence functioning as a sexual signal in extant fireflies (electronic supplementary material, figures S1–S4) [24]. Terrestrial bioluminescence is an ancestral trait in Lampyridae and relatives (Phengodidae and Rhagophthalmidae), preceding the origin of adult aerial bioluminescence that is used widely as a sexual signal in modern fireflies [23,24,80]. Assuming that the original function of bioluminescence was predator deterrence, it begs the question as to what predator, or predators, could have driven the origin of firefly bioluminescence.

(c) Predators

Clade ages for potential predators that fireflies probably encountered during their early history, that would have contributed to the origin of bioluminescence as an aposematic signal, were compiled. We identified potential groups of firefly predators that were hypothesized to feed on these groups and were prevalent and broad enough in distribution to potentially function as significant firefly predators. Terrestrial predators included: Opiliones (harvestmen) [70], Araneae (spiders) [64], Carabidae (carabid beetles) [41], Anura (frog and toads) [67], Squamata (lizards and snakes) [65], Reduviidae (assassin bugs) [69] and Rodentia [68]. Aerial predators included Aves (birds) [71] and Chiroptera (bats) [66]. An estimation for the origins of terrestrial bioluminescence allows us to discuss the likeliness of these predator groups as drivers of the evolution of warning signals. Several of the terrestrial predator groups including Anura, Araneae, Carabidae, Opiliones and Squamata emerged prior to the Jurassic (greater than 200 Ma), before the origin of terrestrial beetle bioluminescence and therefore could have contributed to the origin of aposematic beetle bioluminescence that arose 141 Ma. Reduviidae emerged around 178 Ma; however, most of the diversity in this group did not appear until the Late Cretaceous (approx. 97 Ma) [69].

By contrast, several other insectivore clades (Aves, Chiroptera and Rodentia) originated much later (less than 70 Ma) than beetle bioluminescence and thus could not have been selective agents in either terrestrial or aerial bioluminescence. It has been suggested that modern aerial predators, specifically bats, could have driven the origin of aerial bioluminescence as a predator avoidance strategy

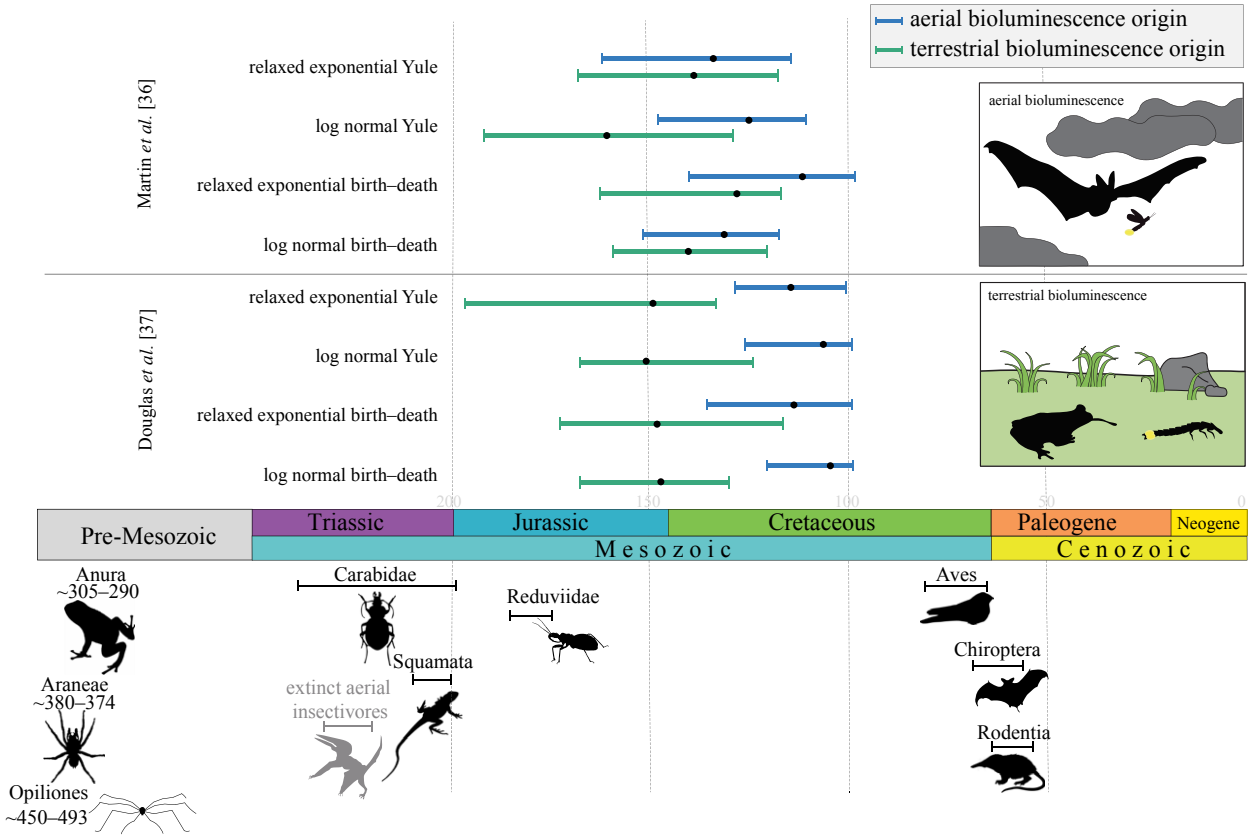

Figure 2. Comparison of divergence time estimates for nodes representing the origins of both terrestrial (green line) and aerial (blue line) bioluminescence and with published clade origin ages for potential extant (black) [41,64–67,69–71] and extinct (grey) predator groups [63]. Times are written out for Opiliones, Anura and Araneae due to age and figure constraints. Dotted vertical lines represent 50 million-year intervals. (Online version in colour.)

[32]. This is contradicted by our data, with bats originating at approximately 65 Ma [66], about 52–87 million years after the origin of aerial bioluminescence in fireflies. This result supports the idea that the original purpose of adult, aerial bioluminesence was that of sexual signalling. However, it is possible that once bioluminescence was used by adult fireflies in aerial displays, potential aerial predators like bats and birds could use adult firefly bioluminescence as an additional cue to avoid distasteful prey [32]. The sensory systems of insectivorous bats can detect both the light spectrum of firefly light emissions and the ultrasonic wingbeat clicks emitted by flying fireflies [81,82]. In addition, bats can learn to discriminate between flying insects based on their different echo signatures in their echolocation calls [83,84], and they make adaptive prey selection decisions to increase profitability [85]. Bats have indeed been shown to reject flying fireflies based on their chemical defences, reinforced by their sonar profile and/or bioluminescent signal that speed up avoidance learning [32]. Whether predators like bats impose selection on the aerial bioluminescence of fireflies and possibly contribute to the maintenance of aerial light signals in beetles remains to be tested. Other predators such as nocturnal birds and rodents [33] that could have preyed on bioluminescent fireflies also originated significantly after the origin of both terrestrial and aerial bioluminescence (figure 2). For example, modern birds originated approximately 75 Ma [71] and rodents at approximately 61 Ma [68], placing them approximately 65–79 million years after the estimated origin of terrestrial bioluminescence and approximately 58–72 million years after aerial bioluminescence (figure 2). Our age estimates for bats, birds and rodents are almost certainly older than the actual insectivorous lineages within each. Given that beetle larval bioluminescence evolved first and operated in a terrestrial environment, aerial predators, such as bats, could not have been the original receivers or drivers of these aposematic signals. The signal would have been directed toward contemporary predators of elateroids in the early Cretaceous.

There are several extinct vertebrate insectivore groups that could have also preyed on lampyrids and other elateroid beetles prior to or during the early Cretaceous (greater than 100 Ma) [86]. Most of the early mammalian insectivores are more limited in both known diversity and distribution in the fossil record [86] and would thus probably have only added to an already existing predation pressure in the terrestrial environment. One potential exception could have been the aerial pterosaurs [87]. Pterosaurs were the first vertebrate group to develop true flight in the late Triassic (approx. 229 Ma), with the common ancestor of the group hypothesized to be insectivorous [88]. Furthermore, Pterosauria had a broad enough distribution to have encountered bioluminescent beetles [87], and some species are assumed to have been crepuscular or even nocturnal [89] and thus could have been early receivers of the aposematic signals of fireflies and other elateroid beetles. However, due to the limitations of the fossil record, we do not have evidence to it confirm that pterosaurs fed on elateroid beetles, and if they did, whether this behaviour was prevalent.

In summary, our extensive taxon sampling and numerous well-placed fossils across each topology, recovered well-supported and congruent ages for bioluminescent beetles. These ages are older than previous estimations and preclude modern aerial predators as selective agents for the origin of beetle bioluminescence. However, several groups of modern

terrestrial predators pre-date the origin of bioluminescence and thus could have functioned as selective agents for the origin of terrestrial bioluminescence in fireflies, and possibly other elateroid beetles. Based on the present-day use and abundance of terrestrial bioluminescence as aposematic signals and the presence of terrestrial insectivores at the origin of beetle bioluminescence strongly suggests that terrestrial beetle bioluminescence arose as an aposematic signal. By contrast, aerial beetle bioluminescence, which is widely used as a sexual signal during mate search in extant beetles, probably originated as such. We do not rule out that extant insectivores such as bats, birds and rodents, that emerged in the fossil record after the origin of firefly bioluminescence, may still operate as contributing factors in maintaining bioluminescence. However, it is now clear that the origin of aerial bioluminescence in fireflies pre-dates any extant aerial predator group. We find that adult firefly bioluminescence outshines, or pre-dates, the origins of extant aerial predators by roughly 60 million years.

Data accessibility. All data analysed in this study are cited in the above manuscript or provided in the electronic supplementary material [90].

Authors' contributions. G.S.P.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft and writing—review and editing; N.A.S.: data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft and writing—review and editing; Y.M.P.: data curation, formal analysis, investigation, validation, writing—original draft and writing—review and editing; K.F.S.-H.: funding acquisition, project administration, validation and writing—review and editing; G.J.M.: data curation, validation and writing—review and editing; D.K.: validation and writing—review and editing; L.F.L.D.S.: data curation, validation and writing—review and editing; L.B.: funding acquisition, project administration, validation and writing—review and editing; M.A.B.: data curation, funding acquisition, project administration, validation and writing—review and editing; S.M.B.: conceptualization, funding acquisition, project administration, visualization, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare that they have no competing interests.

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