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Multiple lineages of lice pass through the K–Pg boundary

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For modern lineages of birds and mammals, few fossils have been found that predate the Cretaceous–Palaeogene (K–Pg) boundary. However, molecular studies using fossil calibrations have shown that many of these lineages existed at that time. Both birds and mammals are parasitized by obligate ectoparasitic lice (Insecta: Phthiraptera), which have shared a long coevolutionary history with their hosts. Evaluating whether many lineages of lice passed through the K–Pg boundary would provide insight into the radiation of their hosts. Using molecular dating techniques, we demonstrate that the major louse suborders began to radiate before the K–Pg boundary. These data lend support to a Cretaceous diversification of many modern bird and mammal lineages.

Keywords: Phthiraptera; evolution; cospeciation; dating

1. INTRODUCTION

The Cretaceous–Palaeogene (K–Pg) boundary 65 million years ago (Ma) was a key episode in recent vertebrate history, marking the end of the dinosaur era and the rise of birds and mammals. This boundary is associated with mass extinctions resulting from an extraterrestrial impact and periods of flood-basalt volcanism [1], the biological consequences of which are still poorly understood and controversial. Of particular interest is how this event affected the diversification of birds and mammals. Some authors have argued that Cretaceous mammalian and avian lineages suffered a mass extinction, and that a period of rapid radiation followed in the Tertiary from a handful of surviving lineages [2–5]. Alternative hypotheses suggest that most modern avian and mammalian orders survived the K–Pg boundary, or that several lineages survived, perhaps on different continents [6–8]. Studies of biogeography, the fossil record and molecular clocks have

each been applied to this question, but the results remain contentious.

One potential source of evidence that has been largely overlooked is the evolutionary history of the parasite fauna infecting modern birds and mammals. Host-specific and fast-evolving parasites represent independent markers that can be used to infer the evolutionary history of their hosts. Many parasites, such as ectoparasitic lice (Insecta: Phthiraptera), show unique adaptations to their hosts acquired during their shared evolutionary history. Many louse taxa show evidence of cospeciation with their hosts and these cospeciation events provide an internal time calibration for estimating the age of the radiation of these parasites [9]. Furthermore, the recent discovery of two important fossils shed light on the age of lice, independent of their hosts. The first is an exceptionally preserved 44 million years (Myr) bird louse fossil, corroborating the antiquity of the bird louse association [10]. The second is the oldest fossil of the book-louse family Liposcelididae (*ca* 100 Ma [11]), which is the closest free-living relative of parasitic lice [12,13]. We use these calibration points, together with data from multiple genetic markers, to establish the age of the major lineages of parasitic lice.

2. MATERIAL AND METHODS

We sampled 69 louse taxa from all four louse suborders (see the electronic supplementary material, appendix for GenBank Accession Numbers). These taxa span eight calibration points, two of which are based on fossils and the remainder derived from cospeciation points that can be reliably dated with evidence from the host fossil record (figure 1). Four hemipteran and one psocopteran taxa were used as outgroups (the psocopteran *Liposcelis decolor* was included in the analysis, but this species is sister to the Amblycera; see below). We sequenced mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 18s rRNA and EF1a genes using published DNA extraction, amplification and sequencing protocols [12,14]. Sequences were aligned by eye, or with the aid of secondary structure data for the 18s rRNA gene. Bayesian phylogenetic analyses were performed in MRBAYES v. 3.12 [15] and divergence times were estimated using two Bayesian dating methods (BEAST [16] and MULTIDIVTIME [17]). Node constraints were assigned uniform prior distributions as shown in figure 1. A user-supplied tree from a Bayesian analysis (MRBAYES v. 3.1 [18]) and a subsequent initial divergence time estimate derived from the chronogram in MULTIDIVTIME [17] was used to initialize BEAST. A detailed account of the analytical procedure is given in the electronic supplementary material.

3. RESULTS

BEAST analysis recovers a chronogram for lice (figure 1) that is consistent with recent studies of phthirapteran phylogeny [12,19]. The monophyly of each louse suborder is generally well-supported and the psocopteran *Liposcelis* is sister taxon to the Amblycera, supporting the polyphyly of Phthiraptera [20]. We calculated the rate covariance among adjacent branches of our data to find evidence of rate autocorrelation. For each gene, the rate of covariance among adjacent branches is very close to 0 (18S, 0.151; EF1, −0.044; COI, −0.054) suggesting there is no strong evidence for autocorrelation of rates [16]. In these circumstances divergence dates calculated with BEAST (without rate-autocorrelation assumption) may be more appropriate than dates from MULTIDIVTIME (based on rate-autocorrelation assumption).

Both BEAST and MULTIDIVTIME gave a Early to Mid-Cretaceous origin (115–130 Myr ago) for extant Phthiraptera (figure 1 and electronic supplementary
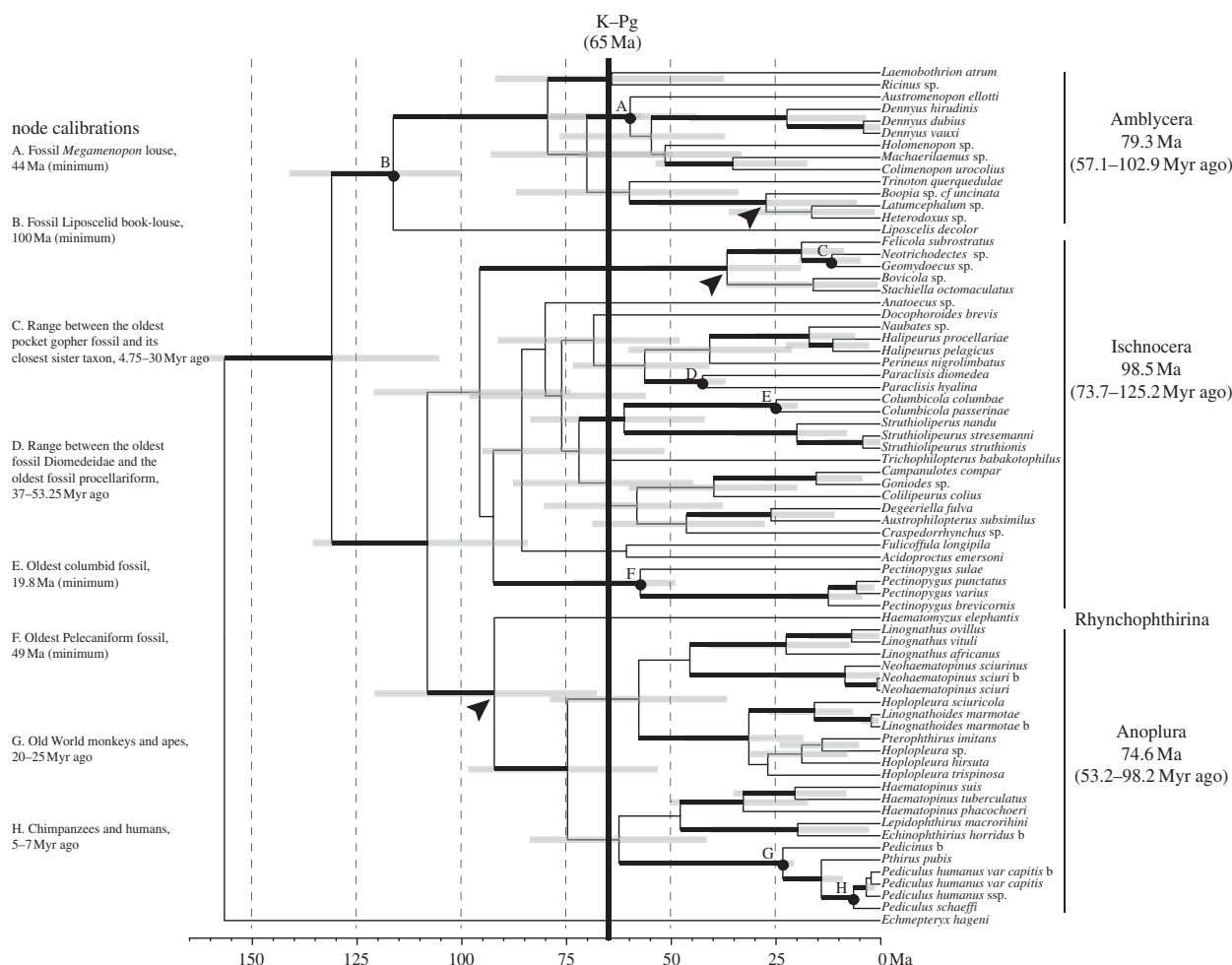
Figure 1. Divergence time chronogram (in millions of years) for Pscoptera and Phthiraptera from analysis of the 3-gene dataset in BEAST. Calibrations are indicated by lettered circles at nodes (see the electronic supplementary material). Grey bars indicate 95% HPD boundaries and thickened branches indicate nodes that received Bayesian posterior probability support greater than or equal to 0.95. Clades parasitizing mammals are indicated with an arrow.

material), with 15–18 lineages of lice from all four sub-orders passing through the K–Pg boundary in each analysis. Although there is a wide margin between the upper and lower bounds of the 95% highest posterior density (HPD) estimates (grey bars in figure 1), no nodal divergence estimates are inconsistent with the age ranges defined by the calibrations.

4. DISCUSSION

This study demonstrates that the major families of lice began to radiate before the K–Pg boundary, and thus supports a Cretaceous diversification of many modern bird and mammal lineages. Our results are consistent with three independent lines of evidence—the insect fossil record, avian and mammalian molecular divergence estimates, and biogeographic data. Recent Mid-Cretaceous finds of fossilized book-louse family ('Psocoptera': Liposcelididae), the close relatives to parasitic lice, led Grimaldi & Engel [11] to estimate that parasitic and non-parasitic louse lineages diverged from each other deep in the Cretaceous, approximately 100–145 Myr ago. Similar dates have been estimated for the radiation of modern bird and mammal orders—the contemporary hosts of parasitic lice

[7,8,21]. The biogeographic evidence from modern mammal and bird groups is also compelling. Phylogenetic studies suggest most basal and extant lineages of birds (e.g. like the ratites) and mammals (e.g. marsupials) have a Gondwanan distribution, leading many to conclude that modern bird and mammal orders had a pre-Tertiary diversification before the final break-up of Gondwana 80–160 Myr ago [22–24].

Another long debated question is whether birds or mammals are the ancestral host for parasitic lice? Simple character state reconstruction across the louse phylogeny infer mammals as the ancestral host of the clade containing Ischnocera, Rhynchophthirina and Anoplura; and birds as the ancestral host for Amblycera. However, reconstructing the alternative host for these clades requires only a single additional step. Other lines of evidence suggest birds may have played a more prominent role in the diversification of lice. Chewing lice of birds are species-rich and ubiquitous; there are approximately 3000 species recognized, parasitizing all but 16 of the 173 extant bird families. This broad distribution across birds coupled with ancient diversification point towards a long history of association with birds. Only Anoplura (sucking lice) appear to have undergone a major radiation on

mammals. The timing of the diversification of sucking lice in the Late Cretaceous (approx. 75 Ma) and subsequent radiation soon after the K–Pg boundary is in agreement with mammalian evolutionary history [7] and a recent study focused solely on the Anoplura [19]. Interestingly, the diversification of extant chewing lice parasitizing mammals postdates the K–Pg boundary, in contrast to the Cretaceous diversification of their avian infesting relatives. This suggests parasitic chewing lice were first acquired by birds and later diversified on mammals. The distribution of lice on mammals also supports this hypothesis. Chewing lice parasitizing mammals are species-poor and patchily distributed across mammalian families. Although anopluran divergence dates suggest a radiation that was largely contemporaneous with their hosts, sucking lice are even more patchily distributed across mammals, parasitizing only 20 per cent of all mammalian species.

Parasitic lice use their host's pelage or plumage as both a habitat and a food resource and host specificity can be attributed to phenotypic variation of host hair and feathers [25,26]. It seems reasonable to assume that the evolution of this fundamental host resource is closely associated with the evolution of parasitic lice. The oldest definitive fossil feathers are still those of *Archaeopteryx* from the Late Jurassic and fossilized hair is not known until the Late Palaeocene (roughly 55 Ma). By this time, both feathers and hair were modern in form and microscopic detail [27], but there is evidence to suggest these characters predate their first definitive occurrence in the fossil record [28,29]. Given the diversification times presented here and the early origin of feathers, parasitic lice probably infested feathered theropod dinosaurs.

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