Early evolution of diurnal habits in owls (Aves, Strigiformes) documented by a new and exquisitely preserved Miocene owl fossil from China

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Nocturnal owls exhibit adaptations thought to be evolutionarily associated with their diets, morphologies (sensory and flight), and diel activity patterns. However, that evolutionary history is not so simple, as demonstrated by an exquisitely preserved partial skeleton of an owl from the late Miocene of China that represents the first fossil evidence for diurnal behavior among owls. The fossil from the high-elevation Liushu Formation preserves most of the skeleton including the scleral ossicles. Osteological features place the holotype specimen as a member of the strigid clade Surniini. In contrast to the largely nocturnal owls, nonnocturnal (diurnal and crepuscular) species are concentrated within the Surniini as a likely evolutionary reversal in diel activity patterns. Analyses of the preserved scleral ossicles in the fossil demonstrate that it exhibits a large exterior scleral ossicle ring diameter with a large orbital length, supporting the hypothesis that this extinct owl was largely diurnal in its habits. Furthermore, stochastic character mapping, combined with Bayesian ancestral state reconstruction of the activity patterns of extant birds, demonstrates higher posterior probabilities of diurnal behavior among early diverging Surniini, and the addition of this extinct taxon into analyses enhances the hypothesis of this clade’s diurnal origin. The fossil and associated analyses of the eye and behavioral evolution point to a long evolutionary history of nonnocturnal behavior among owls that has yet to be studied in detail. This diurnal owl joins a growing Liushu avifauna that would have hunted small mammals in the savanna-like habitats adjacent to the rising Tibetan Plateau.

Strigidae \textsuperscript{a} | Surniini \textsuperscript{b} | diurnal \textsuperscript{c} | Linxia \textsuperscript{d} | fossil \textsuperscript{e}

There is considerable variation in the diel activity patterns across tetrapod taxa and geographic locations and even in those of individuals over time (1, 2), and that degree of variation in activity obfuscates reconstruction of its evolutionary history. The majority of tetrapods are active during daylight hours, and armed with that widespread assessment, the species and groups who are largely active at night have been viewed by most as expressing derived evolutionary states (3). However, the evolution of nocturnality as the predominant diel pattern in species and larger clades conversely has been (controversially) hypothesized as primitive in some cases such as mammals (4–7) and even crown group birds (8). Given the significant amount of behavioral variation present among tetrapods and complications constraining the reconstruction of diel activity preferences from morphology and fossils, research aiming to detail evolutionary changes in activity patterns to or from nocturnality requires multiple lines of evidence from phylogeny and the fossil record (3, 6, 9).

Nocturnal owls have come to be associated in human cultures with a wide variety of motifs from death to luck and wisdom in cultures around the world. With over 200 extant species of owls distributed across all continents (except Antarctica) and many islands (10), owls (Strigiformes) compose the most diverse clade of nocturnal predatory birds. They are important predators in terrestrial ecosystems and impact mostly small mammal communities around the world. Despite their linkage to the night, it should be noted that owls are not exclusively nocturnal, and some species exhibit crepuscular and even diurnal behavior. While owls feature many nocturnal specializations related to hearing and eyesight and have distinct feather modifications allowing for silent flight (11), the evolutionary diversity of their activity patterns has not been assessed in a phylogenetic framework or through the fossil record.

Traditionally, Strigiformes has been allied with the diurnal predatory bird clades Falconidae and Accipitridae (12), but recent phylogenomic analyses support the hypothesis of the independent acquisition of predatory behaviors and diets along with their nocturnal habits (13). That growing phylogenetic framework supports traditional taxonomy that crown group owls are composed of two major extant clades, Tytonidae

Significance

Owls, with their largely nocturnal habits, contrast strikingly with the vast majority of diurnal birds. A new spectacular late Miocene owl skeleton from China unexpectedly preserves the oldest evidence for daytime behavior in owls. The extinct owl is a member of the clade Surniini, which contains most living diurnal owl species. Analysis of the preserved eye bones documents them as consistent with diurnal birds, and phylogenetically constrained character mapping coincides with a reconstruction of an early evolutionary reversal away from nocturnal habits in this owl group. These results support a potential Miocene origin of nonnocturnal habits in a globally distributed owl group, which may be linked to steppe habitat expansion and climatic cooling in the late Miocene.
(barn owls and relatives) and Strigidae (encompassing all remaining species) (14, 15), and the interrelationships among owl genera and groups within Strigidae have been reexamined, resulting in recognition of new groupings and reformulation of other traditional taxa (e.g., Surniini, *Glaucidium* (14, 15). While there is an extensive fossil record of owls of a wide variety of body sizes extending back into the Paleocene, the phylogenetic relationships of the majority of Paleogene fossil taxa with respect to the crown lineages and even the crown group itself are unresolved (16). In the absence of a morphological analysis of owl phylogenetic interrelationships, our knowledge of owl systematics and evolution is limited currently to molecular analyses that lack an internal molecular clock calibration point (14, 15), which only provide hints about the evolution of owl diversity and their phenotypes (such as activity patterns).

Without an internally calibrated owl molecular clock, we must rely on the fossil record to document many aspects of the evolution of crown group owls. The timing of the origin of the crown groups of Strigidae and Tytonidae, along with the major groupings within Strigidae and Tytonidae (tribe and generic level clades) within the Neogene, are largely unconstrained (17). That lack of resolution results, in part, from the nature of the fossil record with the vast majority of fossils known from isolated and disarticulated elements, although with some more complete fossil material from the Paleogene and Pleistocene (16, 18–20). The oldest potential members of crown group Strigidae are represented by fragments from early Miocene of Europe (Mammal Neogene [MN] 2 to 3) and North America, and the oldest definitive tytonid fossil is from the middle Miocene (18, 21). Fossils allocated to the extant genera *Tyto, Asio, Bubo, Strix, Surnia,* and *Glaucidium* and closely related genera are known from a large part of the Neogene (19, 22), and that diversity might represent a Neogene radiation of extant owl groupings. Interestingly, those fossils have been placed in taxa (genera) with extant species that range from fully nocturnal to crepuscular and diurnal, potentially indicating an equal breadth of diel behaviors among owls in the deep past. Mayr (16) proposed that early members of the owl lineage may not have had eyes as well adapted for nocturnal habits as most extant species. Uniquely, fossils can provide morphological evidence for these ancient behaviors through the expression of differences in eye and orbit size and morphology (3), along with the occurrence of some features like a supraorbital process associated with diurnal habits (23).

Owl species exhibiting a predominantly diurnal activity pattern are rare, with only the northern hawk owl (*Surnia ulula*), burrowing owl (*Athene cunicularia*), northern pygmy owl (*Glaucidium californicum*), and short-eared owl (*Asio flammeus*) being active mostly during daylight hours (10). It should be noted that the reported activity patterns of short-eared owl (*Asio flammeus*) are active at night (14, 15). The extant monospecific genus *Surnia* is most closely related to *Glaucidium* (15). While the fossil record of this group is not rich, extending only into the Pleocene and possibly into the Miocene, two extinct species of *Surnia* (*Surnia capeki* and *Surnia robusta*) are known from the Plio-Pleistocene of Europe and Pleocene of Morocco (26–28). The oldest records of *Glaucidium* are similarly from the Pliocene and Pleistocene of North America and Africa (29–31), those of *Athene* extend to the early Pliocene (32), and some fossils from the Miocene of Europe may be related to *Ninox* (18).

A great increase in our knowledge of the Neogene evolution of owls and their ancient activity patterns derives from a spectacularly preserved fossil representing an extinct late Miocene species of diurnal strigid owl, belonging to the clade Surniini. The nearly complete three-dimensional articulated skeleton is from the high-elevation ~6.0 to 9.5 Ma Liushu Formation (in the Linxia Basin adjacent to the Tibetan Plateau at ~2,400 m above sea level), and it includes many rarely preserved aspects such as the scleral ossicles, hyoid apparatus, tracheal rings, sesamoids, ossified tendons, and stomach contents. The exquisite preservation and level of completeness of this individual allow for comprehensive studies not possible on most strigiform fossils. Analyses of the scleral ossicles support a largely diurnal activity pattern (see Results), and the osteological features present in the skeleton position the fossil with the Surniini (morphologically intermediate between *Surnia* and *Glaucidium*). Furthermore, stochastic character mapping along with ancestral state reconstruction of owls points to a likely nonnocturnal origin of the clade Surniini as an evolutionary reversal from the reconstructed nocturnal primitive state for both crown Strigiformes and Strigidae.

**Systematic Paleontology**

The systematic paleontology is as follows:

Aves Linnaeus, 1758

Strigiformes Wagler, 1830

Strigidae Leach, 1820

Surniini Weick, 2006 (33)

*Miosurnia diurna* gen. et sp. nov. Li, Südham, and Zhou, 2022.

**Holotype.** The holotype is Shandong Tianyu Museum of Nature STM 20-1 (Linyi, Shandong Province, China), a nearly complete articulated bird skeleton, lacking the right forelimb and left manual digits.

**Genus and Species Diagnosis.** The taxon is a medium-sized owl with a body length of ~30 cm (estimated from rostrum to pubis; Fig. 1) with an estimated body mass of ~236 to 319 g (34, 35), roughly the same as *S. ulula* and much larger than *Glaucidium* (25). The holotype specimen can be referred to Strigidae using numerous derived features including the presence of an ossified extensorial arcus (retinaculum extensorii) on the proximodorsal surface of the tarsometatarsus (Fig. 1), and *M. diurna* differs from Tytonidae (barn owls) in having a much shorter skull rostrum (~1/3 of skull length; *SI Appendix, Table S1*) and a sternum bearing two notches caudally (Fig. 1). *M. diurna* lacks the irregular projection of the ventrocaudal edge of the naris that is present in strigine genera (*Strix, Asio,* and others) and differs from species of Striginae in the following combination of characters: the mandible bears a short retroarticular process, and the caudal portion of jugal bar bears an enlarged dorsal projection (present in *Surnia, Glaucidium,* and *Ninox*); the projection is rudimentary in *Athene* and also
Fig. 1. Photograph and line drawing of the complete holotype specimen of *M. diurna* (STM 20-1). Anatomical abbreviations: al, alular digit; b, bony residue; c, ceratobranchial; cr, cranial process of sternum; dc, deltopectoral crest; ep, extensor process; er, ossified extensor rectinaculum; ex, extensor sulcus; fe, femur; fi, fibula; fm, foramen magnum; hu, humerus; hy, hypotarsal crest; im, intermetacarpal space; is, ischium; lc, lateral condyle; lt, left tibiotarsus; mc, medial condyle; mm, major metacarpal; mp, manual phalanx; ms, mandibular symphysis; mtll, trochlea of metatarsal II; p, patella; pd, pedal digits; pg, paraglossum; qu, quadrate; ra, radius; rad, radiale; ri, rib; rs, rostrum; rt, right tibiotarsus; sc, scapula; sk, skull; so, scleral ossicle; st, sternum; sy, synsacrum; tm, tarsometatarsus; tr, tracheal ring; ul, ulna; and uln, ulnare.
variable in size within other Surniini taxa (36). The holotype lacks the extremely long and slender zygomatic process present in species of Asioninae (37).

*M. diurna* shares most of its features with taxa within the strigid clade Surniini (i.e., *Surnia* and *Glaucidium*) including a straight minor metacarpal, a short and stocky tarsometatarsus, and particularly an enlarged dorsal projection caudal to the orbit on the caudodorsal portion of jugal bar (*SI Appendix, Fig. S1*). The holotype has a shallower dorsal concave surface of metatarsal trochlea III than that present in *Surnia*. The zygomatic process of the squamosal is pointed and slightly longer than that of *Sur- nia* and *Glaucidium* (*SI Appendix, Fig. S1*). This taxon differs from species of *Glaucidium* in its larger size, proportionally longer rostrum, absence of a bulge on the frontals along the nasal-frontal hinge, and a smaller process on the caudal portion of the jugal bar (37). *S. robusta* is larger and more robust compared with *M. diurna* (26), and metatarsal trochlea IV is positioned more proximal than the state in the holotype. The humerus of *S. robusta* is longer than *M. diurna* with a less projected deltopectoral crest, and the tarsometatarsus of *M. diurna* has a less projected lateral crest adjacent to the cotyle. According to descriptions of the Pleistocene fossil species *S. capeki* (27), the proximal width is greater than in the extant *Surnia* and *M. diurna*. The characters shared between *Miosurnia* and *S. ulula* include a wing-shaped squamosal expansion, six sternal ribs and costal processes, and a knob-like structure projecting ventrally from the minor metacarpal (*SI Appendix, Fig. S1*).

*M. diurna* exhibits two potential autapomorphies. The most caudal free sternal rib (possibly the sixth) has an unusual hammer-shaped outline for articulation with the thoracic rib (*SI Appendix, Fig. S2*), and this morphology is unknown among other species of Strigidae including *Surnia* and *Glaucidium*. The radius of the holotype also lacks the ossified arch that fuses to the proximal end present in most owls (38).

**Age and Horizon.** The *M. diurna* holotype is from the Liushu Formation exposed in the Linxia Basin of Gansu Province, adjacent to the northeastern edge of Tibetan Plateau in China (*SI Appendix, Fig. S3*). The age of the fossil-bearing sediments of the Liushu Formation is 6.0 to 9.5 Ma (39) and roughly equivalent to the European Land Mammal Ages MN 9 to 13 (40).

**Etymology.** The genus name is in reference to its Miocene age and similarity in morphology with the extant owl *Surnia*, and the specific epithet refers to the evidence for this owl as an active diurnal bird.

**Description and Comparisons**

The skull of *Miosurnia* is roughly triangular in shape (ventrally) with an ovoid external narial opening. The size and shape of the naris is similar to that of extant *Athene* and *Surnia* (23). The ventral border of the naris bears a small groove, extending into foramina that penetrate the bone rostrally and caudally. The maxillary part of the palate is rostrally expanded, forming a tongue-like structure. There is an enlarged dorsal process on the jugal bar caudal to the orbit, and it is larger than that of the *S. ulula* but smaller than the size in *Glaucidium* (*SI Appendix, Fig. S1*). The cranial portion of the frontal bone of *M. diurna* is less expanded rostrodorsally than in *Glaucidium*. The area on the dorsal part of the orbit where a supraorbital process occurs in *Surnia* is broken away in the fossil.

The disarticulated ring of scleral ossicles is well ossified and largely fills the orbit. The exterior margin of the lateral ring plate is decorated with small zigzag serrations (Fig. 2). The individual ossicles are slightly concave along their long axis, and they differ from the tubular-shaped ring present among typical nocturnal owls (41). An ossicle’s maximum length is roughly twice its midpoint width, with the outer edge longer...
than the inner edge. The intermetacarpal space widens distally and narrows into a small slit proximally (SI Appendix, Fig. S4). The intermetacarpal space is large and similar to the size to that of extant *Surnia*. The tarsometatarsus is short and stocky with a distinctly medi ally wider proximal end that has a concave medial margin (in contrast to the straighter lateral margin). The three metatarsal trochleae of *Miosurnia* are similar to extant *Surnia* in their distal extension, with metatarsal trochlea IV only slightly shorter than that of the other two (SI Appendix, Fig. S1). Metatarsal trochlea II, III, and IV vary, with the club-shaped trochlea II shorter than III, and the medial margins of the tarsometatarsi are quite convex close to the ossified proximal arcus, as seen in *Bubo, Surnia, and Glaucidium* (SI Appendix, Fig. S1). The medial hypotarsal crest extends quite far plantarily. The club-shaped metatarsal trochlea II is short and stout as in *S. ulula* (SI Appendix, Fig. S1). The foot of *Miosurnia* is clearly zygodactyl, with an extended plantar wing of the fourth metatarsal trochlea rotated toward the midline plantomedially (SI Appendix, Fig. S5). The grasping morphology of the foot also is indicated by the extremely abbreviated proximal pedal phalanges, as compared to the longer distal ones, and large sharp curved pedal unguals (SI Appendix, Table S1). The medial proximodorsal process of the phalanx III-1 extends more distally than the lateral process with the articulation of phalanx III-2, giving it an asymmetrical appearance (SI Appendix, Fig. S5). See SI Appendix for a full description of the fossil specimen.

Results

**Activity Pattern Evaluation** (Phylogenetic Flexible Discriminant Analysis). We used a published dataset (9) (SI Appendix, Table S2) including both avian and squamate eye shapes and sizes (i.e., orbit length, exterior and interior scleral ring diameters, and activity patterns) to discriminate between the eyes of diurnal birds and those of nocturnal and catherinal/crepuscular species (3). We applied phylogenetic flexible discriminant analysis (pFDA) (42, 43) across multiple rounds of analyses and took into account uncertainties related to measurements, reconstructions, and phylogeny. All analyses return statistically higher posterior probability values (pp values) for the classification of *M. diurna* as being a diurnal bird rather than nocturnal (SI Appendix). By plotting the returned discriminant scores 1 and 2 from one round of analysis (Fig. 3) (SI Appendix, Table S3), *M. diurna* is positioned closest to the crepuscular and diurnal *Glaucidium* taxa (e.g., *Glaucidium brasilianum* and *Glaucidium gnoma*) and not far from *S. ulula* (Fig. 3). The two discriminant axes were affected by the optical ratio and eye size (3). In addition, the scleral ring of *Miosurnia* is morphologically similar to other diurnal owls in having both a large exterior scleral ring diameter and a large orbital length, with individual ossicles exhibiting a larger outer edge and smaller inner one (Fig. 2 and SI Appendix, Fig. S6).

**Stochastic Character Mapping and Ancestral State Reconstruction.** We also applied stochastic character mapping using simmap (44) to evaluate the posterior probabilities of internodes as cathernal, diurnal, and nocturnal over one phylogenetic tree used in the pFDA analyses. The posterior probability (pp value) for the node including *Surnia* and *Glaucidium* is calculated to be 99% likely diurnal (extant taxa used only) and with only a 1% chance of it being nocturnal (SI Appendix, Fig. S7 and Table S4). The inclusion of the fossil *M. diurna* into the Surniini clade alters the reconstruction to 100% diurnal posterior probability (Fig. 4).

MrBayes ancestral states reconstruction with R (MBASR) analyses using one tree of the pFDA analyses returned similar results regarding the ancestral state reconstruction of the probability of that same node as being diurnal (node 710, ~0.996) when the fossil was included (SI Appendix, Fig. S8).
probability of each diel activity state for other internal nodes is provided in SI Appendix, Table S5. A second MBASR analysis using the most recent molecular phylogenetic tree of owls (15) produced similar results as well (SI Appendix, Fig. S9). The occurrence of several crepuscular and diurnal taxa within Surniini and their close phylogenetic relationships (15) along with our analyses based on the reconstruction of ancestral eye and behavioral states indicate that a more likely diurnal or crepuscular (not nocturnal) activity pattern was the primitive state for possibly all or part of Surniini.

Independent statistical reconstruction of the ancestral states in both simmap and MBASR across Strigidae for their diel activity patterns points to nocturnality as clearly primitive for crown Strigiformes and Strigidae, and they support a distinct shift to diurnality at the node including Surnia and Glaucidium. The morphology of the scleral ring in M. diurna and its analysis as being consistent with diurnal behavior adds to the different lines of behavioral phylogenetically constrained analyses increasing support in the ancestral state reconstruction (ASR) results for the hypothesis of diurnal behavior as being ancestral for Surniini.

Discussion

Based on morphological examination of the scleral rings (wide with larger outer edges) as well as the results of the pFDA, M. diurna represents documentation of a fossil diurnal owl taxon, and that hypothesis is supported by independent lines of evidence including both discrete morphological comparison and statistical morphometric analysis. The classification probability of the extinct owl by the pFDA is much higher for it to be diurnal (pp values average over 62%) than for it to have been a nocturnal owl, even when taking into account uncertainties in measurements and phylogenetic relationships. In combination with the ASR and stochastic character mapping, a most probable nonnocturnal evolutionary origin of the Surniini (i.e., Surnia + Glaucidium) (see Results and SI Appendix) is well supported, and the occurrence of this diurnal owl in the late Miocene of Asia with fossils of other members of Surniini widely distributed at that time suggests that diurnal (and/or crepuscular) owls may have a longer and more significant history than currently recognized. For example, the known extinct species of Surniini also may have been largely nonnocturnal in their habits. M. diurna may have occupied a niche somewhat similar to that of the extinct sympatric and similarly diurnal kestrel (Falco hezhengensis) consuming small diurnal mammals in the high-elevation savanna-like habitats, but these two birds likely used different foraging strategies (45). The preserved partially digested osteological remains of the individual owl’s unregurgitated final meal show evidence of acid erosion from digestion, and the size and texture of the bony fragments is consistent with that of small mammals (Fig. 5). This preservation parallels that of the fossil kestrel from the Liushu Formation with its jerboa jaw and dental material (45).
unusual owl from the Liushu Formation adds an unexpected component to the growing diversity of birds (with excellent preservation) cutting across ground-dwelling omnivorous galliforms (46), arid associated sandgrouse (47), ostriches (48), and raptorial birds (49). In particular, birds of prey appear to be important components of late Miocene Linxia fauna, including the large-sized carrion feeders (Old World vultures) and medium-sized agile predators (Falco and Miosurnia) (39, 43).

Evolution of Diurnality in Owls. Owls are predominantly nocturnal in their behaviors, but some species are crepuscular and even mainly diurnal. That original evolutionary shift to nocturnality in early owls has had many evolutionary ramifications on the clade including changes to their eye morphology (large rod-dominant eyes) and molecular shifts related to their carnivorous diet and behaviors (50). The large number of olfactory receptors in owls may be related to the greater reliance on smell in nocturnal foraging (50). However, few studies have been conducted on extant diurnal owl species to determine what potential evolutionary reversals or novelties may be present in their evolution. As we can see in the shape and size of owl eyes presented in Results, some aspects of the owl phenotype are plastic enough to readily conform to evolutionary alterations in diel activity patterns, and some workers (41) suggest that diurnal owls have adapted both morphologically and genetically to those behavioral changes. However, adaptation related to the molecular evolution of positively selected genes impacting retinal development (e.g., ALCAM), other aspects of the retina and eye, and related phenotypes in diurnal rather than nocturnal owls (50, 51) has not been fully explored. Detailing the molecular underpinnings of the readaptation to diurnal activity would be a fruitful area of research. As we can see in our study, the morphology of the eye is plastic in owl evolution changing apparently in parallel with their diet activity patterns. Selection for daytime activity likely has had other impacts on the phenotype and genome of nonnocturnal owls.

Furthermore, our analyses of this Miocene fossil as a member of Surniini and as a diurnal species also are suggestive of a long history of diurnality or at least not exclusive nocturnal behavior in this clade of owls. Last, the interpreted paleohabitat of the Liushu Formation is that of an arid savannah (47, 52). Most diurnal owls inhabit open habitats today, and the past environment of the late Miocene of the Linxia Basin is consistent with those types of open habitats being associated with the evolution of diurnal behavior in owls. Based on the preserved shared derived osteological features in the fossil of Miosurnia, it is a member of Surniini and closely related to the clade of Surnia + Glaucidium. This Chinese Linxia fossil also may provide an internal fossil calibration point (minimum age 6.0 Ma) for the origin of the Surniini within Strigidae for future diversification and evolutionary analyses. Differing from the extant distribution of Surnia with its more northerly occurrence, the presence of M. diurna adjacent to the Tibetan Plateau may relate to a southern expansion of typical Palearctic region owls in the late Miocene (~6 Ma) during a period of time with lowered sea level and the growth of polar ice sheets (53).

Materials and Methods

Comparative Materials. Comparative skeletons of owls were examined at the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences, Beijing, China; Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, CA; and National Museum of Natural History (NMNH) (Smithsonian Institution), Washington, DC. Skeletal features for the major five subfamilies/tribes (10) were examined, including major clades: 1) clade Tytoninae (Tyto) and Phodilinae (Phodilus); 2) clade Strigidae (Striginae), Nytet a, Ketupa, Stix, Asio, Otus, and Ciccaba; and 3) clade Surniini, Surnia, Glaucidium, Athene, and Ninax. The fossil specimen was compared closely with extant Surniini taxa and also other species of related to Surniini, including Aegolius and Xenoglaux (15).

The osteological terminology generally follows that of Baumel and Witmer (1993) (54), with English equivalents adopted. Measurements were taken with digital calipers and rounded to the nearest 0.1 mm (SI Appendix, Table S1). Institute abbreviations for specimens are as follows: Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China (IVPP); Museum of Vertebrate Zoology, University of California, Berkeley, CA (MVZ), Shandong Tianyu Museum of Nature, Linyi, Shandong Province, China (STM); USNM, National Museum of Natural History-Ornithology Collection, Smithsonian Institution, Washington, DC.

Body Mass Estimation. We used two methods to estimate the body mass of the holotype individual of the extinct owl M. diurna, with regression equations
using least femur circumference and humeral length (34, 35). With a measured femoral shaft diameter of 3.7 mm and a humeral length of 64.7 mm, the body mass of the extinct owl is estimated using those equations to be between 236 and 319 g.

\[ \log_{10}(\text{estimate}) = 2.411 \times \log_{10}(\text{femoral circumference}) - 0.065, \]
\[ \log_{10}(\text{estimate}) = 1.73 \times \log_{10}(\text{humeral length}) - 0.76. \]

**Phylogenetic Flexible Discriminant Analysis (pFDA).** To infer the diel activity pattern of *Miosurnia*, we performed pFDA on three osteological variables (i.e., orbit length and external and internal diameters of the scleral ossicle ring) following that of ref. 9. Morphometric data including 367 extant species of birds and 55 squashate species derive from ref. 9 with the fossil *M. diuma* holotype included (See Appendix, Table S2). The owl fossil was bound to its most likely phylogenetic position as the sister taxon of the clade (*Surnia + Glaucidium*), diverging from the midpoint of the branch leading to (*Surnia + Glaucidium*), with a tip date set as 6 Ma (according to the youngest age of the Liushu Formation) (56).

We performed a pFDA on species with three sets of measurements, utilizing a phylogenetic framework following the work of ref. 9. All of the analyses followed the same procedure described previously (9). Training data consisted of only extant taxa, with three diel activities that were assigned previously (See Appendix). The phylogenetic uncertainty was taken into account in the pFDA by iterating analyses over 50 phylogenetic trees as in the previous study (9). The three rounds of analyses were performed based on slight differences in measurements of the reconstructed orbit in order to account for any error resulting from incomplete preservation of the fossil scleral ring. Those three analyses should provide the maximum, minimum, and average reconstructed aspects of the eye and ossicle ring and examine if this variation in measurement/reconstruction would alter our results. The resulting pFDA scores (first and second discriminant axes) were used to create Fig. 3, using the package ggplot2 [R package (57)] with the average reconstruction of the orbit and eye dimensions. The code for the pFDA is based primarily on the study of ref. 9 and run in the R environment (58), with the help of other functions from packages ape, class, geiger, picante, and others. The R scripts are available at Open Science Framework (https://osf.io/k46y9/).

**Scleral Ring Reconstruction and Related Measurements.** The scleral ring of *Miosurnia* was reconstructed from the holotype specimen, STNM 20-1. Since some of the individual ossicles are exposed, those measurements were taken and used to reconstruct the complete ring optimized as a circular ring shape resembling extant owls. Available measurements of the individual ring plates were used to optimize the entire circular set based on these individual trapezoidal shapes (16 ossicles combined with an equal change in position around the center as shown in SI Appendix, Fig. S5). Since each ossicle overlaps and is overlapped by the adjacent ossicles along their interior edges, 21.5% of the overlap was deducted from sum of the short edge (inner circle). The number of ossicles is based on the number in *Athena* and other owls, and the amount of ossicle overlap was calculated from our fossil and published images of a cleaned and stained *Athena* ring (59) and extant *Surnia* (USNM 610389). The orbit length was calculated based on constraints in the fossil skull. The caudal rim of the orbit is discernable on the braincase, and the rostral impression of the lacrimal defines the rostral boundary of the orbit. We allowed for uncertainties in its size related to preservation (21.5 to 23.5 mm). Our reconstruction results in an external scleral ring diameter of 23 to 25 mm and an internal diameter of 10.7 to 12 mm (SI Appendix, Fig. S3).

**Discrete Character Evolution and Ancestral State Reconstruction (ASR).** We applied stochastic mapping to the diel activity patterns (dap), using a Markov chain Monte Carlo approach to calculate the probability of each ancestral state (using one of the trees modified from ref. 9). This methodology (44) is used widely to explore ecological data and simulations. The simmap (60) function is embedded in phytool (make.simmap) (61) and run in the R environment (58). We conducted the simmap analysis with nsim = 1,000 and the prior set (pi) as t0.1427, 0.5864, 0.2709 using one of the 50 trees; a prior distribution on the root node for the three states (cathemeral, diurnal, and nocturnal) and default q = empirical applied as a single Q matrix is estimated and used subsequently in each run in phytool (61). A summary of the mapping results returned a calculated posterior probability for each internode and is plotted as a pie chart in Fig. 4 (also see SI Appendix, Fig. S7 and Table S4). The R script is available at Open Science Framework (https://osf.io/k46y9/).

To further evaluate the diel activity pattern evolution in birds, we performed ASR using the MBASR toolkit (54) with one tree from the pFDA analysis, and the results are presented as statistical probabilities of each character state in pie charts for each (ancestral) internode (54). MBASR is an R language toolkit that highly automates the ASR workflow and uses the machinery of the popular phylogenetics software MrBayes (60). The number of simulations was set at 500 with the characters set as unordered. We performed additional round of ASR with a most recently published molecular owl phylogenetic tree (UCE-BI) adopted from ref. 15. The diel activity patterns of extant species of Strigidae are from ref. 10. The activity patterns were coded focusing on the majority pattern of diel activity for each species but with polymorphism allowed (e.g., crepuscular and nocturnal). The fossil taxon *M. diuma* was added to the tree as a sister to the clade *S. ulula + Glaucidium* using the bind tip function in phytools (60), with it added to with the edge length and position set arbitrarily as 0.001 and 0.0005 (SI Appendix).

**Data Availability.** R code has been deposited in the Open Science Framework (https://osf.io/k46y9/). All other study data are included in the article and/or SI Appendix.

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