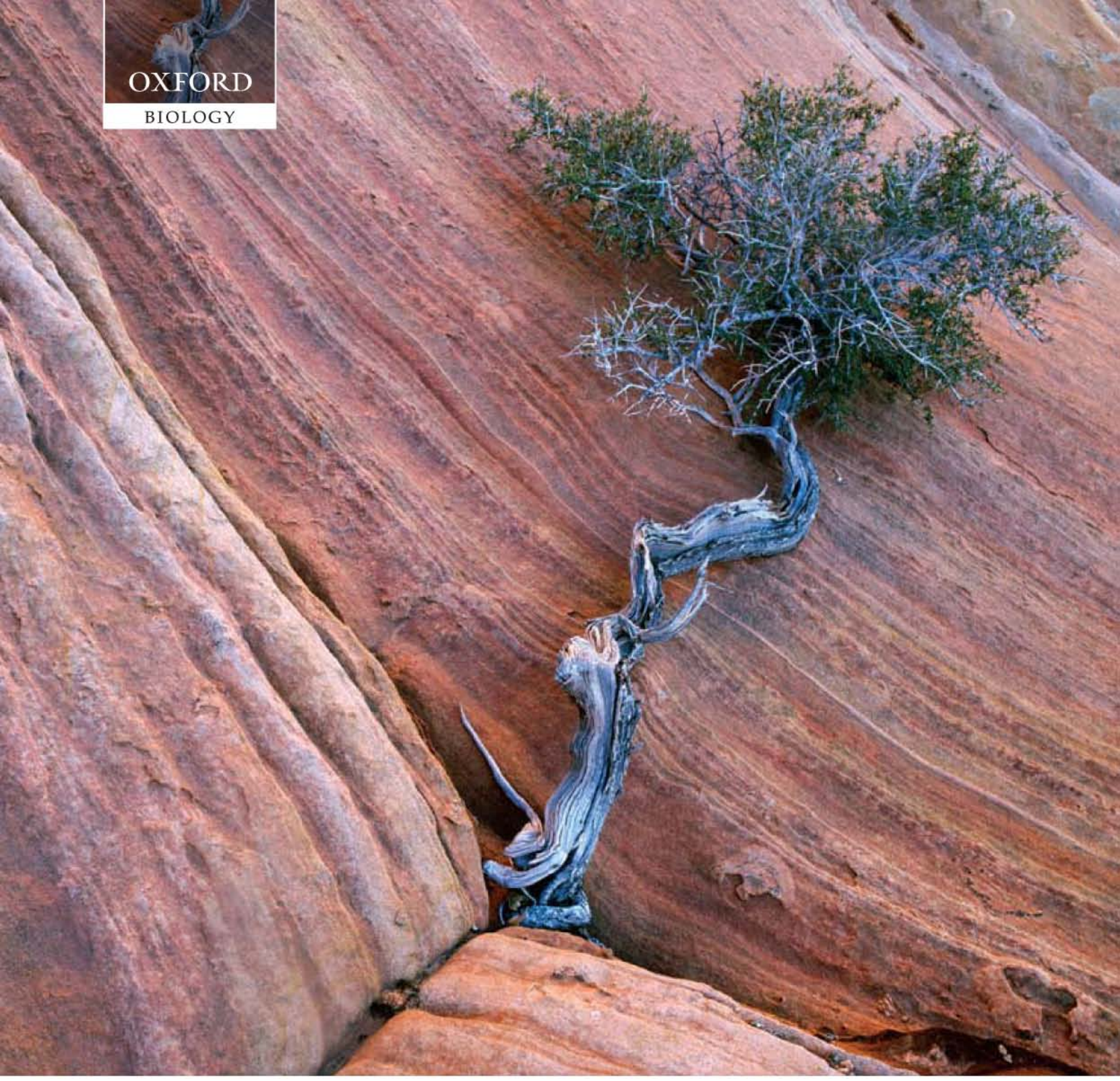

OXFORD
BIOLOGY

the TIMETREE *of* LIFE

edited by **S. BLAIR HEDGES** *and* **SUDHIR KUMAR**

foreword by James D. Watson

Bats (Chiroptera)

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Abstract

Bats are grouped into 17–18 families (>1000 species) within the mammalian Order Chiroptera. Recent phylogenetic analyses of molecular data have reclassified Chiroptera at the interfamilial level. Traditionally, the non-echolocating megabats (Pteropodidae) have been considered to be the earliest diverging lineage of living bats; however, they are now found to be the closest relatives of the echolocating rhinolophoid microbats. Four major groups of echolocating microbats are supported: rhinolophoids, emballonuroids, vespertilionoids, and noctilionoids. The timetree suggests that the earliest divergences among bats occurred ~64 million years ago (Ma) and that the four major microbat lineages were established by 50 Ma.

Bats are nocturnal mammals that have achieved the ability of true self-powered flight and are members of the monophyletic Order Chiroptera (meaning "hand-wing"; Fig. 1). They are the second most species-rich mammalian order (>1000 species) and account for ~20% of all extant mammalian diversity (*1*). They are found throughout the globe and are only absent from the extreme polar regions, but some bat lineages show high levels of endemism (*1*). Bats exploit many environmental niches and can feed on insects, fish, fruit, pollen, nectar, mammals, birds, and blood. They are important pollinators and play an important role in the tropical ecosystems (*2*).

There are two major types of bats: megabats and microbats. As the names suggests, the largest bats are megabats (40–220 cm wingspan) and the smallest bats are microbats (22–135 cm wingspan). Another major difference between these groups is their mode of sensory perception (*3, 4*). Microbats (17 families) are capable of using sophisticated laryngeal echolocation, whereby they acoustically perceive their environment by interpreting returning echoes of emitted sound (*5*). In contrast, megabats (one family) rely on large eyes specialized for nocturnal vision (*4*). *Icaronycteris index* is one of the oldest bat fossils (~55 Ma) and is considered a microbat; however, the majority of the bat fossil record is fragmentary and missing key species (*6, 7*). Here I review the relationships and divergence times of the extant families of bats.

Traditionally bats have been divided into two superordinal groups: Megachiroptera and Microchiroptera (see *8, 9* for reviews). Megachiroptera was considered basal and contained the Old World megabat family Pteropodidae, whereas Microchiroptera contained the 17 microbat families (*8, 9*). Although this division was based mainly on morphological and paleontological data, it highlighted the difference in mode of sensory perception between megabats and microbats. Because all microbats are capable of sophisticated laryngeal echolocation whereas megabats are not (*5*), it was believed that laryngeal echolocation had a single origin in the lineage leading to microbats (*10*). The 17 families of microbats have been subsequently divided into two infraorders, Yinochiroptera (rhinolophids, hipposiderids, megadermatids, craseonycterids, rhinopomatids, emballonurids, and nycterids) and Yangochiroptera

Fig. 1 An Old World leaf-nosed bat (*Hipposideros larvatus*), Family Rhinolophidae, in flight from the Kanchanaburi region in Thailand. Credit: S. Puechmaille.

E. C. Teeling. Bats (Chiroptera). Pp. 499–503 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).

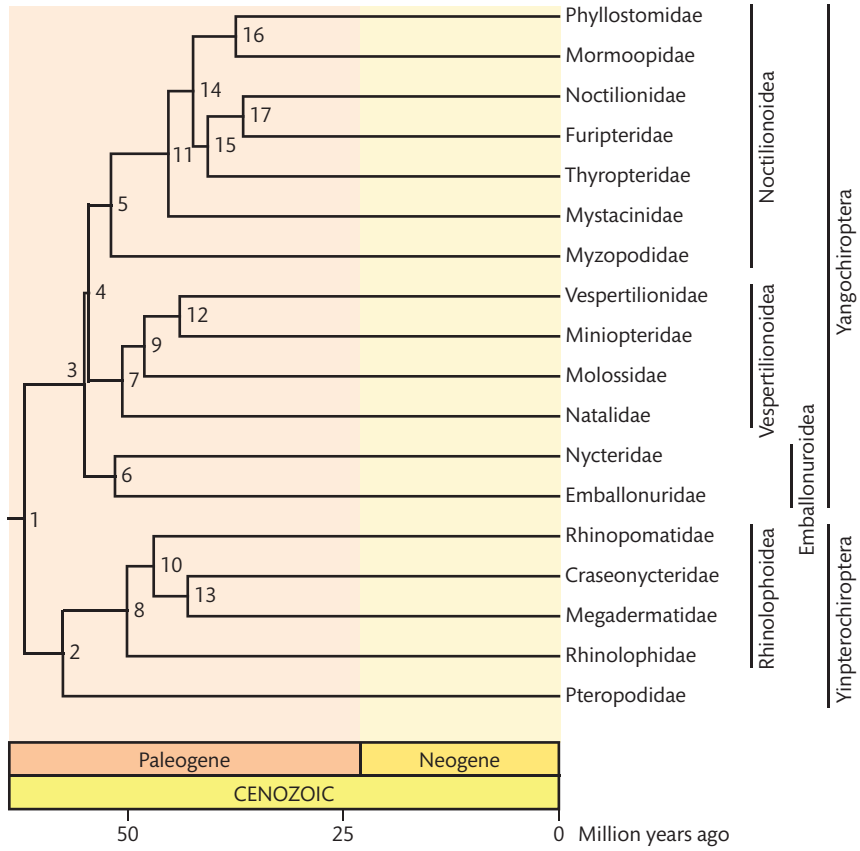

Fig. 2 A timetree of bats (Chiroptera). Divergence times are shown in Table 1.

(vespertilionids, molossids, natalids, phyllostomids, noctilionids, furipterids, thyropterids, mormoopids, mystacinids, and myzopodids), based on whether their premaxillaries were moveable/absent or fused relative to their maxillaries (*8, 9, 11*). This arrangement was largely supported by recent morphological data sets (*6*) and supertree consensus studies (*12*). However, the number of superfamilial groupings varied in content and number among studies (*6, 8, 9, 12*).

From the onset it became apparent that molecular data did not support the monophyly of Microchiroptera and, therefore, did not support a single origin of laryngeal echolocation. Rather, molecular data supported a basal division between Yinpterochiroptera (rhinolophoid microbats and pteropodids) and Yangochiroptera (all other bats; *7, 13–16*). This topology suggested that laryngeal echolocation either originated in the ancestor of all bats and was subsequently lost in lineages leading to the megabats or originated more than once in the microbat lineages (*10*). Initially immunological distance data (*17*); single gene data sets (*18, 19*); whole genomic DNA–DNA hybridization studies (*20*); repetitive genomic elements (*21*); and taxonomically limited consensus studies (*22*) all supported microbat paraphyly to different degrees (*4*). However, strong support and congruence for the association of the rhinolophoid microbats with the pteropodids was only derived from large concatenated nuclear data sets with representatives from nearly all putative bat families (*7, 13, 14*) and rare cytogenetic signature events (*23*).

Molecular data in the form of large nuclear and mitochondrial concatenations provide strong support for the association of four major groups of echolocating microbat lineages (Fig. 2): (a) Rhinolophoidea, which includes the rhinolophids (which includes the hipposiderinids), rhinopomatids, craseonycterids, and megadermatids (*7, 13–15*); (b) Emballonuroidea, which includes the nycterids and emballonurids (*7, 13–15*); (c) Vespertilionoidea, which includes the vespertilionids, molossids, natalids, and miniopterids (*7, 13–15*); and (d) Noctilionoidea, which includes the noctilionids, phyllostomids, furipterids, thyropterids, mormoopids, mystacinids, and

Table 1. Divergence times (Ma) among bats and their credibility/confidence intervals (CI) among bats (Chiroptera).

Timetree		Estimates							
Node	Time	Ref. (*7*)		Ref. (*13*)		Ref. (*14*)		Ref. (*30*)	
		Time	CI	Time	CI	Time	CI	Time	CI
1	62.0	64	71–58	62	70–56	64	70–58	57.9	–
2	57.5	58	63–53	58	65–52	58	63–53	55.8	–
3	55.0	55	61–50	53	61–47	56	62–50	56	–
4	54.5	54	60–50	–	–	55	61–50	54.6	–
5	51.9	52	57–46	–	–	52	57–46	51.6	–
6	51.5	52	58–47	50	57–43	52	58–47	52.1	–
7	50.6	50	56–45	50	58–44	51	56–45	51.4	–
8	50.1	52	55–48	54	60–48	52	55–48	42.5	–
9	48.1	47	53–42	48	56–42	48	54–43	49.3	–
10	47.0	49	53–45	50	54–44	50	54–46	39	–
11	45.3	46	51–41	44	51–37	45	51–41	46.1	–
12	44.0	–	–	45	53–39	43	49–38	–	–
13	43.0	43	47–39	–	–	43	47–39	–	–
14	42.4	42	47–37	–	–	41	47–36	44.3	–
15	40.7	40	46–35	–	–	40	45–36	42.1	–
16	37.5	36	42–32	39	46–33	36	41–31	38.8	–
17	36.6	36	41–31	39	46–33	35	41–30	36.2	–

Note: Node times in the timetree represent the mean of time estimates from different studies. Number of genes analyzed are 17 (*7*), 16 (*14*), and 4 (*13*).

myzopopdids (*7, 14*). The position of Myzopodidae was not resolved by nuclear intronic or mitochondrial data (*14, 15*). Also, the relationships within Noctilionoidea differed when analyzed with nuclear introns (*13*) vs. either nuclear exons (*7, 14*) or mitochondrial data (*15*).

Within these superfamilial groups, molecular data also has revealed novel interfamilial relationships with unique biogeographic and morphological implications. The grouping of Koopman's yinochiropteran Families (*8*) Emballonuridae and Nycteridae with the other yangochiropteran families indicates that the unique mammalian condition of moveable premaxillaries must have arisen at least twice in bats (*7, 11*). Rhinolophoid microbats are united by the presence of pubic nipples, which are not found in any other bat lineage. All rhinolophoids uniquely posses an ossified first costal cartilage fused to the manubrium and first rib (*9, 13*). While it is believed that this structure may reduce the energetic costs of stationary echolocation emission and, therefore, may be evidence for a dual origin of laryngeal echolocation in bats (*13, 24*), this has been questioned (*4*). Within the Superfamily Noctilionoidae, the monotypic New Zealand Family Mystacinidae is the closest relative of the Neotropical noctilionoid families and the monotypic Malagasy myzopodids are the earliest diverging lineage (*7, 14*). This topology suggests a previous Gondwanan distribution and perhaps vicariant origin; however, molecular dates indicate that the Noctilionoidea started to diversify long after the separation of the Gondwanan supercontinent (*7, 13, 14*). Molecular data support the elevation of miniopterids to familial status and suggests that they are closest relative of Vespertilionidae rather than a member of that family (*13–15, 25*). This change in rank is also supported by a unique suite of morphological characters (see *14, 25* for reviews) and deep divergence dates (45 Ma; *13, 14, 25*).

One of the first estimates of bat divergence dates was based on a concatenated data set of five nuclear and three mitochondrial genes and used a quartet dating approach (*26*). Due to limited taxonomic sampling, only the

divergence time of the earliest split among living bats was estimated at 54–52 Ma (*26*). Three recent, large molecular studies have estimated the divergence dates for crown-group bat families (*7, 13, 14*). These dates are all based on a relaxed Bayesian clock method (*27, 28*) with similar constraints and priors available from the fossil record (*7, 13, 14*). Teeling *et al.* (*7*) used a concatenation of 13.7 kb from fragments of 17 nuclear genes (exons and untranslated regions) as representative of all bat putative bat families. Miniopteridae, which was considered a subfamily at the time, was not included. The data set included 30 bat genera and four laurasiatherian outgroups (*29*). Six fossil constraints were employed: (a) a maximum of 34 Ma for the base of the Family Phyllostomidae; (b) a minimum of 30 Ma for the Mormoopidae/Phyllostomidae split; (c) a minimum of 37 Ma for the split between Vespertilionidae/Molossidae; (d) a minimum of 37 Ma for the base of Emballonuridae; (e) a minimum of 37 Ma for the base of Rhinolophidae; and (f) a maximum of 55 Ma for the base of Rhinolophoidea. Bayesian dating analyses (*27, 28*) were used to estimate the branch lengths and divergence times for the entire concatenation and also for each gene considered as a unique partition within the data set. The earliest split among living bats was estimated to have occurred ~64 Ma at or following the Cretaceous–Paleogene boundary (Fig. 2). The four major echolocating microbat lineages all originated within a narrow time frame (~52–50 Ma) within the early Eocene. All extant bat families were estimated to have diversified by the end of the Eocene (~34 Ma). Crown-group pteropodids did not originate until the early Oligocene (28 Ma); however, they had diverged from the rhinolophoids by the late Paleocene (~56 Ma) (Fig. 2).

The data set of Eick *et al.* (*13*) was based on a concatenation of 4 kb of DNA sequence from four nuclear introns for 17 of the 18 bat families (including Miniopteridae, but missing Craseonycteridae) for 55 bats and three laurasiatherian outgroups. Eick *et al.* (*13*) recovered a highly congruent topology with Teeling *et al.* (*7*); however, they reported a close relationship between Mystacinidae and Thyropteridae, a basal position for Myzopodidae within the Vespertilionoidea and a grouping of the Vespertilionoidea with the Noctilionoidea, although these alternate groupings received little bootstrap support (*13*). Like Teeling *et al.* (*7*) they incorporated Bayesian dating analyses with constraints from the fossil record, but only analyzed the concatenation as a single partition. Similar to Teeling *et al.* (*7*) they jackknifed fossil constraints, and found time estimates to be robust to use of different fossil constraints. The dating results from Eick *et al.* (*13*) and Teeling *et al.* (*7*) were nearly identical (Table 1). Miniopteridae is estimated to have diverged from the Vespertilionidae at 45 Ma (Fig. 2, Table 1).

The most recent estimate of interfamilial divergence times is an augmentation of the Teeling *et al.* (*7*) data set to include an additional basal representative of Vespertilionidae and two miniopterid species (*14*). The data set included 11 kb of DNA fragments from 16 genes (*VWF* is not included in this data set). The entire concatenation was analyzed as a single partition using Bayesian methods, with constraints from the fossil record as incorporated by Teeling *et al.* (*7*). The results were similar to previous divergence dates (Table 1). Miniopteridae is estimated to have diverged ~45 Ma (Fig 2; Table 1). These molecular results (Table 1) are corroborated by an independent dating analysis with larger taxonomic scope (*30*). The authors estimated the relative molecular dates for each node by fitting sequence data from six genes to a supertree consensus topology (*12*) and that of Teeling *et al.* (*7*). They incorporated local clocks, which were calibrated by nodal ages extracted from the fossil record and/or previously published absolute molecular dates (*30*).

The timetree suggests that the four major lineages of echolocating microbats originated within a narrow time frame (~52–50 Ma). This was coincident with an ~7°C rise in the global temperature (Paleocene/Eocene thermal maximum), a significant increase in plant diversity and the peak of tertiary insect diversity (*7*). These dates imply that the major echolocating microbat lineages may have radiated in response to an increase in prey diversity and roost sites (*7*). Jones *et al.* (*30*) also reported an increase in bat diversification, particularly the phyllostomids, at 40–25 Ma, which correlates with an increase in flowering plant diversity. This suggests that phyllostomid bats may have radiated due to an increase in fruit and pollen food sources.

Although the earliest divergence among living bats is highly supported (64 Ma), the geographic location is still contentious with biogeographic analyses of similar molecular topologies suggesting either Africa (*13*) or North America (*7*). This is partly attributed to a poor fossil record. Indeed, Teeling *et al.* (*7*) compared the oldest fossil dates with the molecular estimates for each branch on the tree and suggested that the fossil record underestimates first occurrences by on average 73%. They also suggested that at least 98% of fossil history is missing from the megabat lineages. Perhaps this explains the difficulty in assessing whether laryngeal echolocation was lost in the megabat lineages or never acquired in

the first place. Stem megabat fossils, which may or may not show a gradual change in skull structure resulting from a switch in sensory perception (auditory–visual), are not found. Although the timetree has revolutionized our understanding of bat evolutionary history we are still not able to determine whether laryngeal echolocation evolved once or more in bats. Future comparative genomic studies are needed to establish the molecular mechanisms that underlie laryngeal echolocation. This would enable researchers to assess if all laryngeal echolocators are governed by the same molecular mechanisms (which would indicate a single origin of echolocation in bats) or not.

Likewise, it is pertinent that we keep searching for key transition fossils that will shed light on the evolution of echolocation. The most basal bat fossil has only recently been found (*Onychonycteris finneyi*) and shows evidence of flight but not laryngeal echolocation capabilities (*31*). This fossil has enabled biologists to determine that flight most likely originated in bats before the ability to echolocate and answer the long standing question of which came first, flight or echolocation. More fossils of this nature are needed, indeed the timetree and inferred biogeographic hypotheses could suggest new areas and time transects to target for future fossil discoveries.

Acknowledgment

This work was supported by a PIYRA Science Foundation Ireland grant.

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