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ORIGINAL PAPER

Evolution of Traditional Aerodynamic Variables in Bats (Mammalia: Chiroptera) within a Comprehensive Phylogenetic Framework

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Abstract

Chiroptera is the only group of mammals capable of powered flight. The mechanical basis of bat flight is well established, but evolution of its constituent aerodynamic variables remains poorly understood. Here, we determine the macroevolutionary patterns of traditional aerodynamic variables (wing loading, aspect ratio, tip shape index) in a comprehensive phylogeny of Chiroptera using an extensive dataset including key Eocene fossils. We optimized variables as continuous characters and fit models of character evolution to identify shifts in character optima. The reconstructed ancestral chiropteran morphotype presented low wing loading and low-to-intermediate aspect ratio, and remained unaltered for much of the first half of bat evolution (Paleogene). This evolutionary pattern may be explained by stabilizing selection responding to the strong constraints imposed by echolocation and flight on body size, and the physical constraints regarding aerodynamic efficiency acting on wing shape. Posterior specialization in some groups permitted divergence toward novel aerodynamic morphotypes in the second half of chiropteran evolutionary history (Neogene). We linked the most notable aerodynamic changes to ecological release from echolocation constraints (Pteropodidae), dietary-foraging shifts (Phyllostomidae, Noctilionidae), or advantage in face of environmental changes (Molossidae, Taphozoinae). The independently-evolved specialization of fast, enduring flight that allowed Molossidae and Taphozoinae (Emballonuridae) to perform aerial hawking of swarming insects in open spaces was linked to significant shifts in the optima of both wing loading and aspect ratio. These shifts were probably associated with the gradual spread of open-mosaic landscapes at a global scale since the Oligocene.

Keywords Phylogeny · Macroevolutionary patterns · Wing loading · Aspect ratio · Tip shape index · Constraints

Introduction

Chiroptera is the second most diverse group of mammals after Rodentia, with ca. 1400 recognized extant species (Simmons and Cirranello 2018). The great diversity early achieved by this lineage (Smith et al. 2012) is probably related to its capacity of powered flight, unique among mammals. The airfoil in bats consists of a set of compliant wing membranes called "patagia" (Norberg 1972, 1990; Swartz et al. 1996; Song et al. 2008; Waldman and Breuer 2017), mainly supported by the elongated bones of the forelimbs but also by the modified hind limbs. The aerodynamic basis of flight in bats is relatively well understood (e.g., Norberg 1990). Recent studies have focused on the vortex theory, which considers the unsteady aerodynamic effects, i.e., both the time history of the wing movement as revealed by the wake vortices, as well as the forces that accelerate the air around the wing (Rayner 1986; Tian et al. 2006; Hedenström et al. 2007; Muijres et al. 2008, 2012a;

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Hedenström and Johansson 2015). These aerodynamic models show the complexity of vertebrate powered flight and, particularly, of the multi-articulated bat airfoil with >20 mobile joints (with joint reduction presented in some clades; Norberg 1990; Bahlman et al. 2016). However, these models have only been applied to a limited set of taxa (e.g., Tian et al. 2006; Muijres et al. 2012a). By contrast, traditional studies have focused on the variation of wing morphology across a wide sample of bat species, and seek to understand the link between flight style and foraging strategy within a morphofunctional framework (e.g., Vaughan 1966; Lawlor 1973; Norberg 1981, 1986; Aldridge and Rautenbach 1987; Norberg and Rayner 1987; Norberg and Fenton 1988; McKenzie et al. 1995; Norberg et al. 2000; Bullen and McKenzie 2001; Swartz et al. 2003; Hodgkison et al. 2004; Voigt and Holderied 2012; Tavares 2014). Particularly, Norberg and Rayner (1987) sought to clarify the functional bases of eco-morphological correlations in bats, using a dataset of 257 extant bat species belonging to 16 families.

From this perspective, wing morphology can be broadly described by three main derived variables (Norberg and Rayner 1987). Wing loading (WL) is calculated as W / S, where W is the body weight and S is the surface of the aerofoil. The former (measured in pascals [Pa]) is positively correlated with the square of velocity; i.e., higher WL requires a faster flight in order to produce enough lift, resulting in a less maneuverable flight (Norberg and Rayner 1987; Pennycuick 1989; Norberg 1990, 1994). Aspect ratio (AR) is adimensional and describes wing shape; AR is defined as B / C, where B is wingspan and C is the average wing chord (i.e., the mean distance between leading and trailing edges along the wing). High AR wings dissipate less useful aerodynamic energy (lift) through drag-producing wingtip shedding (McGowan 1999). In this way, aerodynamic efficiency (Lift-to-Drag ratio) is maximized, reducing induced power (Norberg and Rayner 1987; Pennycuick 1989; Norberg 1990, 1994). A third variable, the tip shape index (I_{tip}), describes wingtip geometry (Norberg and Rayner 1987), and is determined by the relative size between the handwing and the armwing; $I_{tip} > 1$ indicates rounded to nearly square wingtips, as in most bat species; $I_{tip} = 1$ denotes a roughly triangular shape; and $I_{tip} < 1$ indicates sharper wingtips (Norberg and Rayner 1987; Norberg 1994). I_{tip} correlates with agility across the flight speed range. In slow-flying bats, agility is obtained through wide wings with rounded wingtips (i.e., low AR, high I_{tip}); in high-speed bats, agility is obtained from narrow wings with sharper wingtips (i.e., high AR, low I_{tip}; Norberg 1994). Together, these three main aerodynamic variables are very useful to broadly describe and compare the aerodynamic performance of bat species for which detailed biomechanical studies are still lacking.

Macroevolution of aerodynamic variables in Chiroptera has not been yet studied within an explicit phylogenetic framework. Here, we aim to determine the macroevolutionary patterns of the main aerodynamic variables in Chiroptera using a comprehensive phylogeny. We relied on traditional aerodynamic variables, for which a considerable bulk of data is currently available, because of its widely recognized comparative value. The functional significance of the reconstructed aerodynamic macroevolutionary changes is discussed within the form-function paradigm (Radinsky 1987; Kay this volume).

Materials and Methods

Aerodynamic Data

We used our previous dataset (Amador et al. 2019) that consisted of six descriptive aerodynamic variables compiled from the literature; we chose only those studies that took measurements and estimated variables as in Norberg and Rayner (1987) to maintain data comparability across sources. Three were primary variables (Fig. 1): wingspan (B), as the distance from tip to tip with fully extended wings; wing area (S), measured as the total surface of both wings, the body surface in-between, and the uropatagial surface; and body mass (BM). The remaining three were derived variables: wing loading (WL), as the ratio between W (body mass times gravity acceleration) and S; aspect ratio (AR), estimated practically as the square of B divided by S; and the tip shape index (I_{tip}), calculated as in Norberg and Rayner (1987; Fig. 1). When a particular datum was missing for a species, we calculated it from the remaining known variables. Our original dataset was pruned in order to match the taxonomic sampling of the phylogeny used (see below). Thus, the final dataset included 378 extant bat species, representing 19 out of 21 currently recognized families, plus eight fossil bat species that belong in five extinct bat families with records from the Eocene (ESM_1). The dataset had a variable degree of species representation both among variables, with I_{tip} having the greatest proportion of missing data, and among taxa (Table 1).

Phylogenetic Framework

For parsimony analyses (see below), we used a pruned version of our phylogenetic hypothesis (Amador et al. 2018) including 378 species (out of 800+ included in Amador et al. 2018). We added to the pruned tree eight stem Eocene bat species: †*Onychonycteris finneyi* (†Onychonycteridae), †*Icaronycteris index* (†Icaronycteridae), †*Archaeonycteris trigonodon,* †*A. pollex* (†Archaeonycteridae), †*Hassianycteris messelensis,* †*H. magna* (†Hassianycteridae), †*Paleochiropteryx tupaiodon*, and † *P. spiegeli* (†Palaeochiropterygidae), following the topology of Simmons et al. (2008), with *O. finneyi* sister to all bats, and the other

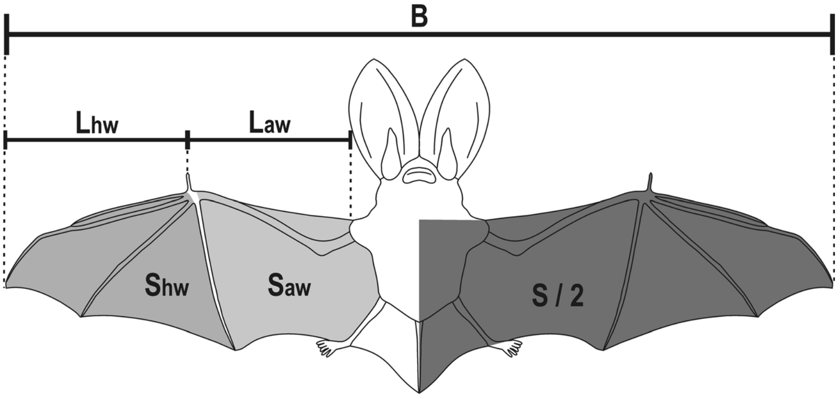


Fig. 1 Primary measurements used in the literature following Norberg and Rayner (1987) to calculate the derived aerodynamic variables. The primary variables are: wingspan (B); wing area (S), both total wing area as that corresponding to each the armwing (S_{aw}) and the handwing (S_{hw}); length of the armwing (L_{aw}) and length of the handwing (L_{hw}). From these basic variables, previous authors calculated the derived variables: wing loading (WL) as body weight (W) divided by S; aspect ratio as the square of B divided by S; and the tip shape index (I_{tip}) as $I_{tip} = T_s / (T_l - T_s)$, where T_s is the tip area ratio between handwing and armwing (S_{hw} / S_{aw}), and T_l is the tip length ratio (L_{hw} / L_{aw})

Eocene taxa as successive sister groups of crown Chiroptera (Figs. 2 and 3). For the Bayesian analyses (see below), we pruned the dated phylogeny of Amador et al. (2018) in order to get a dataset without missing data for each aerodynamic variable.

Evolution of Aerodynamic Variables

First, we evaluated the potential phylogenetic signal in aerodynamic variables using the "phylosig" function in the *phytools* package (Revell 2012) run in R version 3.5.0 (R Core Team 2018). We used both Blomberg's K and Pagel's λ, which suggested a significant degree of phylogenetic signal for all variables, except for I_{tip} (both methods) and BM (body mass) (Blomberg's K; not shown), so we set out to determine the macro-evolutionary patterns of aerodynamic variables in Chiroptera. We applied a local, node-by-node descriptive approach within a parsimony framework using TNT software (Goloboff et al. 2008). We mapped each aerodynamic variable onto the phylogeny as a continuous character (see Goloboff et al. 2006), with a single value (the species average) per terminal for each character. We interpreted nodal location, sign (increase or decrease), and magnitude of net change, the latter defined as the amount of increase or decrease for a given variable at a given branch that is common to all optimal character reconstructions (Giannini 2012; Amador and Giannini 2016; Moyers-Arévalo et al. 2018; Abello et al. 2018). Then, we quantified the frequency and magnitude of net increases and decreases along the overall phylogeny, as well as within the most speciose families. We compared the magnitude of net change among bat families with different taxonomic richness by dividing the total magnitude of net change by the number of species with data in our matrix for each clade.

Second, we tested standard models of trait evolution (Brownian Motion, BM; Ornstein–Uhlenbeck, OU; and Early Burst, EB) available in the *geiger* package (Harmon et al. 2007). We applied the Akaike weight information criterion for model selection. The best fitting model for all

Table 1 Taxonomic sampling of each aerodynamic variable for Chiroptera as a whole and for the seven most speciose bat families. Abbreviations: WL: wing loading; AR: aspect ratio; I_{tip}: tip shape index; B: wingspan; S: wing area; BM: body mass

Clades	Included species	WL		AR		I_{tip}		B		S		BM	
		Species with data	%	Species with data	%	Species with data	%	Species with data	%	Species with data	%	Species with data	%
Chiroptera*	386	321	83.2	319	82.6	198	51.3	376	97.4	319	82.6	383	99.2
Pteropodidae	54	33	61.1	33	61.1	20	37.0	53	98.1	33	61.1	53	98.1
Hipposideridae	23	21	91.3	21	91.3	13	56.5	23	100.0	21	91.3	23	100.0
Rhinolophidae	32	29	90.6	29	90.6	18	56.3	32	100.0	29	90.6	32	100.0
Emballonuridae	21	11	52.4	11	52.4	4	19.0	20	95.2	11	52.4	21	100.0
Phyllostomidae	49	48	98.0	49	100.0	34	69.4	48	98.0	48	98.0	49	100.0
Molossidae	30	26	86.7	25	83.3	8	26.7	28	93.3	25	83.3	29	96.7
Vespertilionidae	131	110	84.0	109	83.2	72	55.0	127	96.9	109	83.2	130	99.2

*including fossils

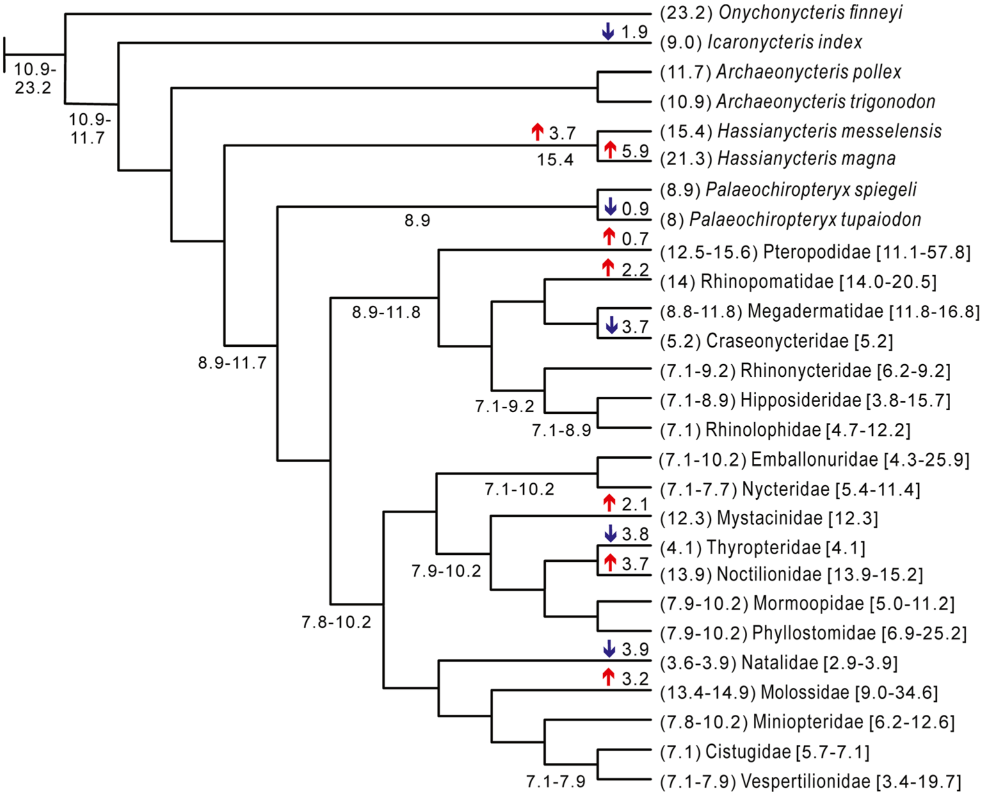


Fig. 2 Parsimony optimization of wing loading in the backbone of the chiropteran pruned phylogeny of Amador et al. (2018), including basal extinct taxa placed following Simmons et al. (2008). Arrows denote net increases and decreases. Ancestral reconstructed values are indicated below branches only when they changed. Wing loading values (Pa) indicated between parentheses correspond to the estimated values for extinct bats or to the reconstructed values for each extant family ancestor. The square brackets contain the interval of observed values at each family

aerodynamic variables was OU (not shown); therefore, we explored further this evolutionary model in our data using a Bayesian approach implemented in the package *bayou* (Uyeda et al. 2014) to identify possible shifts in phenotypic optimum (here aerodynamic variables) along a phylogeny (Uyeda and Harmon 2014). This method does not require predetermined regimes and is free of biases identified in the likelihood approach (see Cooper et al. 2016). A MCMC chain using default, relaxed priors with three million generations was conducted. Parameters were sampled every 1000 generations and results were summarized after discarding the first 750 samples (25%) as burn-in. A second run with the same characteristics was done to check for convergence, which was done using the Gelman and Rubin's R statistics and plots of branch posterior probabilities. For each run, the effective sample size of each parameter was evaluated so that the minimum was 150.

Results and Discussion

Ancestral Morphotype and its Maintenance along the Chiropteran Backbone

The adding of basal extinct species in the parsimony analyses allowed us to reconstruct the early chiropteran evolution of aerodynamic variables, as well as the aerodynamic morphotype of crown-ancestor. These results from parsimony optimizations are summarized in Tables 2, 3, Figs. 2 and 3, ESM_2, and ESM_3. A displacement of the reconstructed WL interval was apparent along the nodes from the stem- to crown-ancestor of bats, from 10.9–23.2 Pa to 8.9–11.7 Pa (Fig. 2). This displacement was caused mainly by a reduction in BM from 23–40 g to 14–16 g (ESM_3), which was accompanied only by a little decrease in S, changing from 173 cm^2 to 149–155 cm^2 (ESM_3). The change in WL must have occurred rapidly, i.e., during the ca. 5 my separating the stem and crown chiropteran ancestors (Amador et al. 2018). We argue that strong directional selective forces during this time resulted in considerable flight refinements. Slow and more maneuverable flight was possible through lowering WL (Norberg and Rayner 1987; Norberg 1994; Amador et al. 2019). Besides, echolocation, an energy-demanding navigation system, evolved at the same time and likely also played a role in shaping this evolutionary change. Sophisticated laryngeal echolocation appeared in full bloom in the next node up the *Onychonycteris* branch (Simmons et al. 2008), imposing an additional constraint on body size during early bat evolution, caused by the inverse scaling relationship between size and sound frequency variables (Jones 1999; Moyers-Arévalo et al. 2018).

Similar to the observed pattern in WL, a decrease in B, albeit slight, was reconstructed through the basal nodes leading to a value of 29 cm for the crown bat ancestor (near the bat median: 30 cm; ESM_3). Along with this change, a subtle increment in AR was reconstructed, from 5–6.1 to 6.4–6.5

Table 2 Results from wing loading parsimony optimization for Chiroptera as a whole and for the seven most speciose bat families. Frequencies of net increases, net decreases, and stasis - ambiguous changes are indicated, both in absolute terms (#) and as a percentage (%) relative to the total number of nodes of the pruned tree. Magnitude of increases and decreases are indicated as well, both in absolute terms (#) and as a percentage (%) relative to the total magnitude of net changes (in Pa). In the last column, the total magnitude of net change relative to clade size is calculated, with the highest values in bold

Clades	Frequency						Total number of nodes	Magnitude (Pa)				Total net change (Pa)	Total net change / # species with data
	Increases		Decreases		Stasis + Ambiguity			Increases		Decreases			
	#	%	#	%	#	%		#	%	#	%		
Chiroptera*	100	13.0	95	12.3	575	74.7	770	262.6	67.1	−128.8	32.9	391.4	1.22
Pteropodidae	10	9.3	6	5.6	91	85.0	107	47.4	75.1	−15.7	24.9	63.1	**1.91**
Hipposideridae	6	13.3	6	13.3	33	73.3	45	7.5	57.3	−5.6	42.7	13.1	0.62
Rhinolophidae	10	15.9	8	12.7	45	71.4	63	15.5	75.6	−5.0	24.4	20.5	0.71
Emballonuridae	3	7.3	2	4.9	36	87.8	41	9.5	84.8	−1.7	15.2	11.2	1.02
Phyllostomidae	18	18.6	15	15.5	64	66.0	97	35.6	58.0	−25.8	42.0	61.4	1.28
Molossidae	8	13.6	9	15.3	42	71.2	59	52.6	73.7	−18.8	26.3	71.4	**2.75**
Vespertilionidae	33	12.6	35	13.4	193	73.9	261	59.2	64.9	−32.0	35.1	**91.2**	0.83

*excluding root

(bat median: 6.4, Fig. 3). The I_{tip} interval reconstructed for the crown-ancestor was 1.38–1.54 (ESM_3). All these features, taken together, indicate a chiropteran crown-ancestor morphotype having wings with low WL, low-to-intermediate AR, and rounded wingtips. This morphology would permit a slow, maneuverable, agile flight, close to or within vegetation, thus resembling the wing morphology and flight style of most of the extant vespertilionids. This aerodynamic performance, together with a small body size consistent with an echolocation frequency sufficiently high, would have enabled bats to access the nocturnal insectivorous aerial-hawking niche, so characteristic of most bats. This reconstruction fit the extinct bat *Palaeochiropteryx*; indeed, the reconstructed values for the chiropteran crown-ancestor were set in the previous node, *Palaeochiropteryx* + extant bats (Figs. 2 and 3 and ESM_3). These values remained unchanged from this node up to all the crown backbone branches, even up to the ancestor of the most speciose family, Vespertilionidae. We attribute this lack of observable change to evolutionary stasis produced by strong stabilizing selection that would have taken place during the ca. 30 my elapsed from the origin of bats to the origin of Vespertilionidae (dated phylogeny in Amador

Table 3 Results from aspect ratio parsimony optimization for Chiroptera as a whole and for the seven most speciose bat families. Frequencies of net increases, net decreases, and stasis - ambiguous changes are indicated, both in absolute terms (#) and as a percentage (%) relative to the total number of nodes of the pruned tree. Magnitude of increases and decreases are indicated as well, both in absolute terms (#) and as a percentage (%) relative to the total magnitude of net changes. In the last column, the total magnitude of net change relative to clade size is calculated, with the highest values in bold

Clades	Frequency						Total number of nodes	Magnitude				Total net change	Total net change / # species with data
	Increases		Decreases		Stasis + Ambiguity			Increases		Decreases			
	#	%	#	%	#	%		#	%	#	%		
Chiroptera*	93	12.1	89	11.6	588	76.4	770	60.4	64.6	−33.1	35.4	93.5	0.29
Pteropodidae	10	9.3	9	8.4	88	82.2	107	8.2	77.4	−2.4	22.6	10.6	**0.32**
Hipposideridae	6	13.3	7	15.6	32	71.1	45	1.8	31.0	−4.0	69.0	5.8	0.28
Rhinolophidae	7	11.1	12	19.0	44	69.8	63	2.1	30.4	−4.8	69.6	6.9	0.24
Emballonuridae	3	7.3	0	–	38	92.7	41	2.3	100.0	0.0	–	2.3	0.21
Phyllostomidae	17	17.5	13	13.4	67	69.1	97	8.9	68.5	−4.1	31.5	13.0	0.27
Molossidae	9	15.3	4	6.8	46	78.0	59	10.5	86.8	−1.6	13.2	12.1	**0.48**
Vespertilionidae	29	11.1	36	13.8	196	75.1	261	16.5	56.3	−12.8	43.7	**29.3**	0.27

*excluding root

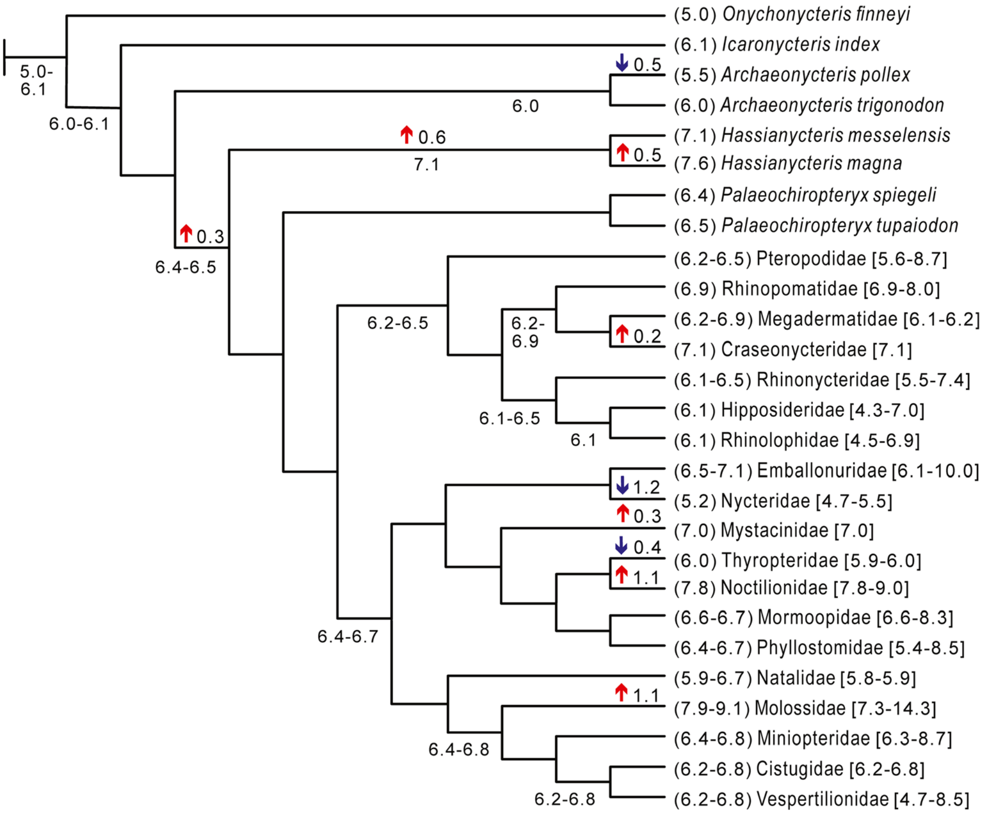


Fig. 3 Parsimony optimization of aspect ratio in the backbone of the chiropteran pruned phylogeny of Amador et al. (2018), including basal extinct taxa placed following Simmons et al. (2008). Arrows denote net increases and decreases. Ancestral reconstructed values are indicated below branches only when they changed. Aspect ratio values indicated between parentheses correspond to the estimated values for extinct bats or to the reconstructed values for each extant family ancestor. The square brackets contain the interval of observed values at each family

et al. 2018). Less than half of the ancestral nodes of families had net changes (in parsimony context) or optimum shifts (in Bayesian context) across all variables, i.e., most changes were located within families (Figs. 1, 2, 3, 4, 5, ESM_3, ESM_4; see below).

Thus, bats seemed to have reached an optimum value for each aerodynamic variable (as it is indicated by the favored OU model), which remained unaltered for much of the first half of bat evolution; diversification and specialization in some particular groups permitted divergence toward novel morphotypes of bat flight in the second half of chiropteran evolutionary history.

Evolutionary Changes of Aerodynamic Variables within Bat Families and their Functional Interpretation

Aerodynamic evolution in bats can be grossly described as variation along morpho-functional axes in the space defined by WL, AR, and I_{tip}. However, evolutionary changes did not occur evenly along these three axes. WL presented the greatest proportion of optimum shifts in the Bayesian framework (Fig. 4). WL depends on body size in two ways, directly as an allometric variable, and indirectly through the effect of tridimensional size on bidimensional wing surface. Shifts toward higher WL values, as well as those in the primary variables BM, B, and S, were concentrated in Pteropodidae (ESM_4), the Old World fruit bats. Pteropodidae includes frugivorous and nectarivorous bats, and is the only bat family that has lost the capacity of echolocation (Simmons et al. 2008, 2010; Wang et al. 2017). It is hypothesized that pteropodids experienced an ecological release from constraints imposed by echolocation on body size (Moyers-Arévalo et al. 2018) and on size-dependent aerodynamic variables. Thus, body size in pteropodids is exclusively constrained by the physiology of flight; so maximum body size might not exceed a relatively low threshold calculated to be ca. 2 kg (Norberg and Norberg 2012; Moyers-Arévalo et al. 2018). The pteropodid species that experienced the most notable WL increases were large species (e.g., *Hypsignathus monstrosus*) and commuting and migrating species, which are favored by a very fast flight (e.g., *Eidolon helvum*, *Pteropus* spp.; Norberg 1990; Neuweiler 2000). These species cover impressive distances in a day that are unreachable for other mammals of comparable size. Shifts in WL optima were also reconstructed in Molossidae, Taphozoine (Old World emballonurids), Rhinopomatidae, and some Vespertilionidae (e.g., *Nyctalus* spp.). All these insectivorous aerial-hawking groups are composed of exceptionally fast and agile species that forage above the canopy or in wide open spaces (Norberg and Rayner 1987). Additionally, *Nyctalus* contains migratory species (Schober and Grimmberger 1997).

Moderate increases in WL optima were observed in Noctilionidae and Phyllostomidae (Fig. 4). Noctilionidae

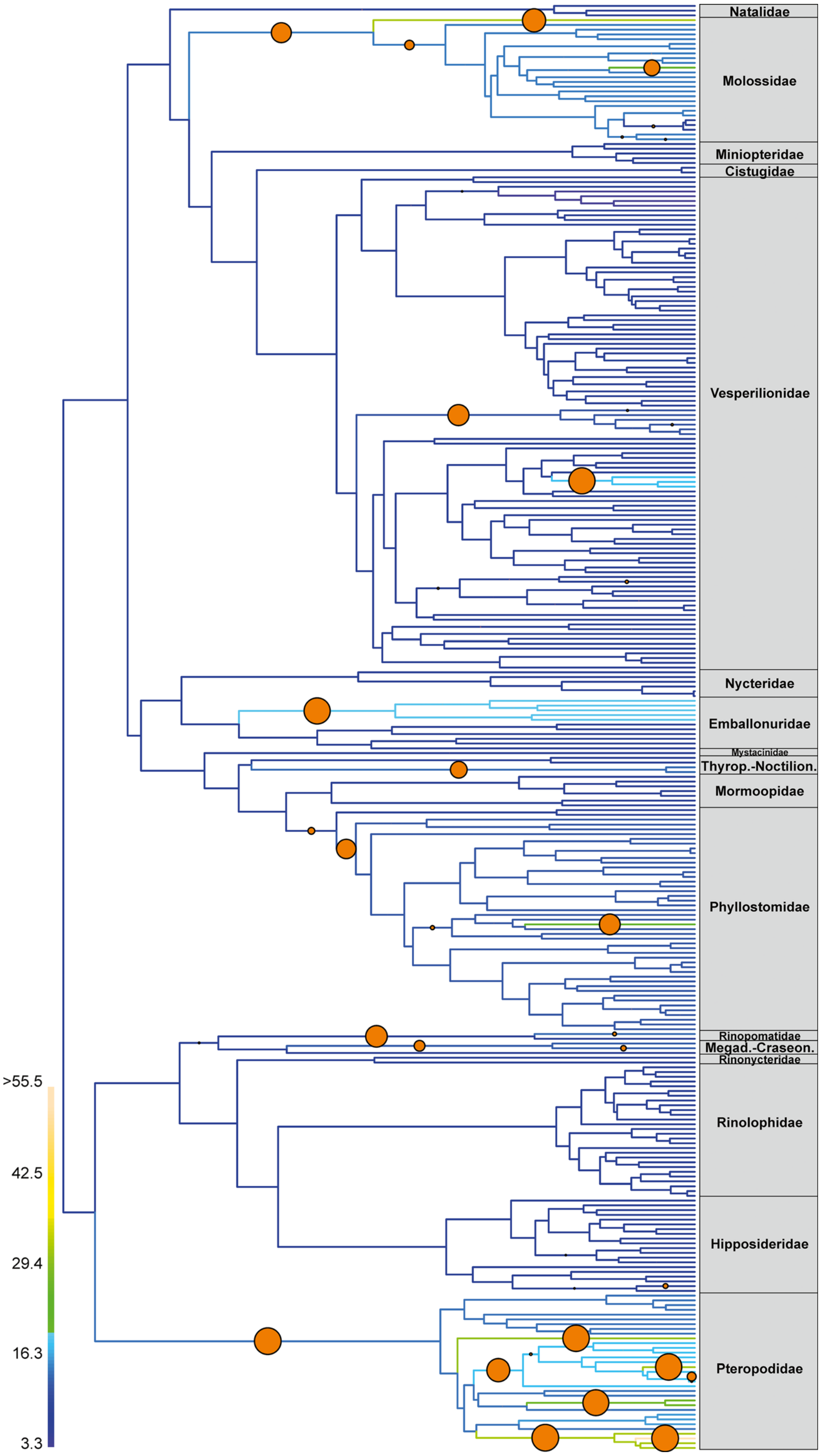
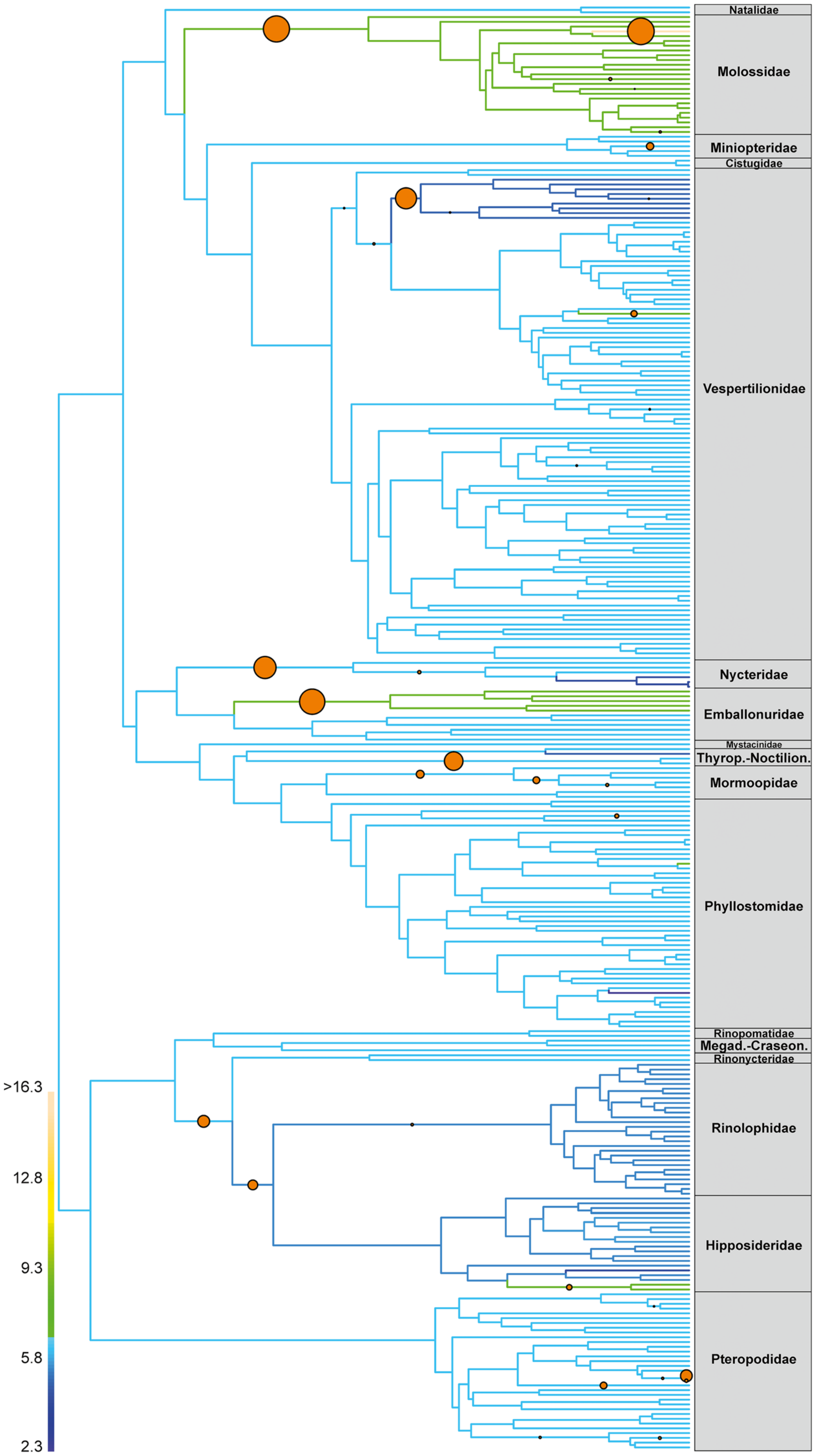


Fig. 4 Bayesian analyses for wing loading (WL). The circles indicate possible shifts in the WL optimum according to an OU evolutionary process, with the circle size corresponding to the probability of each shift. The colored heat map is overlapped to show the direction of the shifts (i.e., increases or decreases). Abbreviations.: Thyrop.: Thyropteridae; Noctilion.: Noctilionidae; Megad.: Megadermatidae; Craseon.: Craseonycteridae

includes narrowly specialized trawling bats that catch fish and insects from the water surface; by contrast, phyllostomids have evolved a wide spectrum of dietary specializations, including frugivory, nectarivory, carnivory, and sanguivory (Neuweiler 2000). These specializations likely evolved within a context of ecological opportunity by means of strong directional selective forces that have led to the wide cranial diversification known in phyllostomids (Rossoni et al. 2017). These morphological specializations have not been restricted to the skull; in fact, some changes in wing morphology must have been necessary for bats to access the new dietary resources. Interestingly, the greatest shift in WL optimum within Phyllostomidae, albeit modest in magnitude, was reconstructed in the branch leading to the large *Phyllostomus*

Fig. 5 Bayesian analyses for aspect ratio (AR). The circles indicate possible shifts in the AR optimum according an OU evolutionary process, with the circle size corresponding to the probability of each shift. The colored heat map is overlapped to show the direction of the shifts (i.e., increases or decreases). Abbreviations.: Thyrop.: Thyropteridae; Noctilion.: Noctilionidae; Megad.: Megadermatidae; Craseon.: Craseonycteridae



hastatus, an incredibly versatile species and one of the few bats with truly omnivorous diet (Giannini and Kalko 2005).

AR and I_{tip} are adimensional variables describing the shape of the total wing and the wingtips, respectively; these variables may be constrained by physical limitations related to aerodynamic efficiency. During postnatal development, bats grow their wings to their final shape (maximum AR) before the young reaches the average adult size (e.g., Stern et al. 1997; Elangovan et al. 2004; Wang et al. 2014). The onset of flight occurs when final AR is achieved, although wing digits have not acquired their definite length yet. Significant AR increases were restricted to clades that simultaneously increased WL: Molossidae and Taphozoinae (Fig. 5; ESM_5); the fast flight produced by high WL becomes more efficient when it is accompanied by a high AR. These clades independently evolved a new style of enduring, long distance, long duration flight, and conquered a new foraging microhabitat: aerial hawking of swarming insects in open space far above the ground and away from vegetation (Vaughan 1966; Voigt and Holderied 2012). Molossidae and Emballonuridae were the only two bat families that presented an allometric pattern of change in AR (Norberg and Rayner 1987), suggesting an adaptive process. Interestingly, these clades diversified immediately after the Eocene-Oligocene boundary (31.5 mya in Molossidae and 30.2 mya in Taphozoinae; crown-ages from dated phylogeny in Amador et al. 2018). This age was characterized by a global-scale increase in aridity that resulted in a retreat of the previously ubiquitous Paleogene forests; members of the more derived Poaceae subclades spread, giving way to the gradual appearance of an open-mosaic landscape (Strömberg 2011; Bouchenak-Khelladi et al. 2014). Although the grass-dominated habitats will have to wait until the middle Miocene for C_3 or late Miocene / early Pliocene for C_4 grasslands, with some intercontinental differences (Strömberg 2011), a mosaic landscape that included both forests and open woodland / grassland elements would characterize the end of the Paleogene (Strömberg 2011; Bouchenak-Khelladi et al. 2014). This environment was a likely scenario for the convergent evolution of these open-space, fast-flying chiropteran clades (Molossidae and Taphozoinae) that differentiated from most insectivorous bats, which hunt close or within vegetation, such as vespertilionoids and rhinolophoids. In developmental grounds, AR increases in these clades could have occurred by the relative lengthening of digit III (influencing wingspan) over digit V (influencing wing chord); differential expression of bone morphogenetic proteins (BMPs) might explain this pattern, as it is known that upregulated BMP2 explains exceptional digital lengthening in bats as compared with a mouse model (Sears et al. 2006). By contrast, decreases in AR optima were reconstructed in Nycteridae, and in the clade of Kerivoulinae + Murininae (Vespertilionidae; Fig. 5). These clades include species that catch insects in cluttered spaces within vegetation (Norberg 1994), similar to the foliage gleaning phyllostomids (e.g., *Macrotus* and *Micronycteris*), a foraging strategy that requires short wings to enhance maneuverability but also low WL (high S through wide wings) for a slow flight.

Finally, I_{tip} tended to concentrate optimum shifts on more terminal branches (Fig. 6; ESM_5); increases were expressed within Hipposideridae, Rhinolophidae, Phyllostomidae, and Vespertilionidae, particularly in gleaning bats that forage in cluttered spaces (e.g., *Chrotopterus auritus*). These slow fliers have increased their agility evolving more rounded or rectangular wings. The terminal shifts shown by the I_{tip} variation seemed to reflect relatively recent adaptations of certain taxa belonging in families that did not experience substantial changes in the other two aerodynamic variables. These particular changes in wingtip shape might have permitted bats to exploit further the gleaning foraging style.

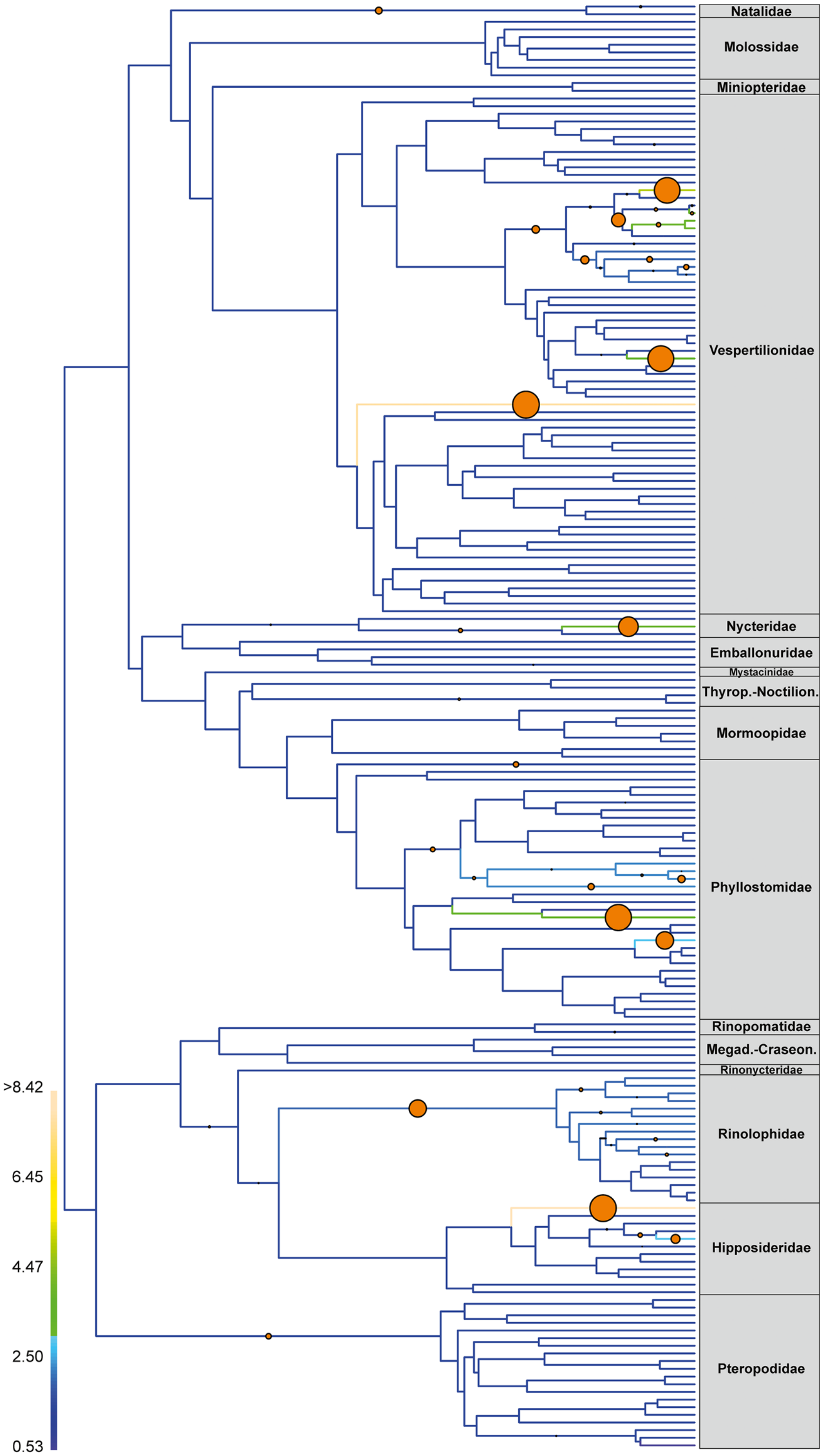
Magnitude of Change

The total magnitude of net changes accumulated in parsimony optimization across our 386-terminals phylogeny, considering both increases and decreases, were: 391.4 Pa for WL, 93.5 units for AR, 44.64 units for I_{tip}, 12.97 m for B, 106.9 dm^2 for S, and ca. 5 Kg for BM (Tables 1 and 2; ESM_2). Pteropodidae concentrated ca. 68% of the total magnitude of change in BM, and ca. 30% in B, whereas Vespertilionidae is the family with the greatest accumulation of changes in the remaining aerodynamic variables (Tables 1 and 2; ESM_2). Notably, Vespertilionidae is the family with the highest taxonomic richness. When we compared the magnitude of net change relative to clade size, this ratio was highest in BM, B, and S in Pteropodidae, I_{tip} in Hipposideridae, and WL and AR in Molossidae (Tables 1 and 2; ESM_2), matching the Bayesian results. Interestingly, the most speciose bat family, Vespertilionidae, was greatly conservative regarding relative (richness-corrected) magnitude of net change in all aerodynamic variables. Thus, this family largely maintained the ancestral morphotype, the successful aerial-hawking foraging model aided by frequency-modulated echolocation. The most notable changes occurred in other insectivorous bat families that exploited alternative foraging microhabitats, perhaps avoiding overlap with the cosmopolitan Vespertilionidae.

Morphofunctional Space of Bats in a Comparative Context

According to our results (see also Aldridge and Rautenbach 1987; Norberg and Rayner 1987; Norberg 1994; McKenzie et al. 1995; Bullen and McKenzie 2001; Hodgkison et al. 2004), the aero-ecological chiropteran niche could be roughly divided into three major morphofunctional zones: 1. Low-to-intermediate WL and low-to-intermediate AR wings characterizing bat gleaners and slow aerial hawkers, as in the case of most of Vespertilionidae, Phyllostomidae, Rhinolophidae, and Hipposideridae, which mostly maintained the ancestral chiropteran morphotype, with only subtle changes in WL or I_{tip}; 2. high WL and low-to-intermediate AR wings defining the commuting / migratory non-echolocating bats (i.e., some pteropodid species) as well as Rhinopomatidae and some vespertilionids that hawk insects in open areas at low height; and 3. high WL and high AR wings that distinguish the agile and fast aerial hawkers hunting insects in open spaces at large distances above the ground, i.e., the highly specialized Molossidae and Taphozoinae (see Fig. 3 in Bullen and

Fig. 6 Bayesian analyses for tip shape index (I_{tip}). The circles indicate possible shifts in the I_{tip} optimum according an OU evolutionary process, with the circle size corresponding to the probability of each shift. The colored heat map is overlapped to show the direction of the shifts (i.e., increases or decreases). Abbreviations.: Thyrop.: Thyropteridae; Noctilion.: Noctilionidae; Megad.: Megadermatidae; Craseon.: Craseonycteridae



McKenzie 2001). Therefore, with the exception of the latter, echolocating bats do not seem to differ substantially among themselves in gross aerodynamic variables, although they might differ in other, potentially significant variables not evaluated in this study. The most frequent changes occurred in allometric variables (i.e., affected by size), with the magnitude of these changes constrained by flight and echolocation (see Moyers-Arévalo et al. 2018 and citations therein). Therefore, niche differentiation in Chiroptera was more related to food selection and dietary specializations accompanied by cranial and dental changes (e.g., Freeman 1998; Swartz et al. 2003; Dumont 2007; Santana et al. 2011; Tavares 2014; Rossoni et al. 2017), and/or diversification in echolocation parameters (e.g., Neuweiler 1989; Maltby et al. 2010), than to fundamental changes in flight style. Moreover, bats have roughly maintained the proportion of digits supporting the wings during the past 50 my (Sears et al. 2006).

In a wider comparative context, the realized aerodynamic morphospace of bats appears smaller than that of birds, or even pterosaurs, considering limb dimensions (McGowan and Dyke 2007). This indicates a relatively lower variation in wing morphology and, concomitantly, flight styles in bats. McGowan and Dyke (2007) pointed out that bird evolution of the granivore guild (e.g., landfowl and tinamous) and secondary flightlessness (e.g., ostrich, rheas, moas, kiwis) are among the possible causes of the disparity in morphospaces seen between birds and bats. Additionally, birds seem to outperform bats regarding aerodynamic efficiency due to phylogenetic constraints that resulted in two different wing designs; birds generate more body lift than bats, and have an aerodynamically inactive upstroke, whereas bats produce thrust and negative lift during upstroke (Muijres et al. 2012b). The differences in flight performance may help to explain why bats are generally slower flyers, and migrate less often and over shorter distances than birds (Muijres et al. 2012b). Another key difference between these two groups of vertebrate flyers is related to reproductive traits. Unlike birds, most bat species carry their offspring during flight, so this additional mass increases WL in the female, raising the aerodynamic power required to fly, and compromising maneuverability; echolocating bats with higher WL are less able to compensate for additional mass, so they exhibit proportionally smaller litter mass (Hayssen and Kunz 1996).

Conclusions

The strong constraints that echolocation and flight have imposed on the evolutionary expression of body size in bats, as well as the physical constraints regarding aerodynamic efficiency acting on wing shape, have modeled an ancestral aerodynamic morphotype that was maintained throughout most of the chiropteran diversification. We infer that this paucity of changes, which involved even the most speciose and cosmopolitan bat family, Vespertilionidae, are the reflection of strong stabilizing selective forces. The realized morphospace of bats is reduced even when it is compared with other flying vertebrates, like birds, denoting the presence of additional constraints (e.g., reproductive, physiological, or morphological). In a scenario dominated by evolutionary stasis, the most notable changes reconstructed in aerodynamic variables were related to ecological release from echolocation constraints (Pteropodidae), dietary-foraging shifts (Phyllostomidae, Noctilionidae), or environmental opportunities (Molossidae, Taphozoinae). These changes could be explained by punctual shifts from stabilizing selection to directional selection towards new optima. In spite of the seemingly very strong phylogenetic and aerodynamic constraints affecting bat wing evolution, this exceptional group of mammals was the first to become truly cosmopolitan, diversifying into numerous (>1400 extant) species able to exploit a considerable diversity of ecological resources.

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Data Availability All data generated or analyzed during this study are included in this published article and its supplementary information files (see ESM_1).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Comment on Ethics This article does not contain any studies with human participants or animals performed by any of the authors.

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