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Convergent Anuran Middle Ear Loss Lacks a Universal, Adaptive Explanation

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Abstract

Introduction: Shared selection pressures often explain convergent trait loss, yet anurans (frogs and toads) have lost their middle ears at least 38 times with no obvious shared selection pressures unifying "earless" taxa. Anuran tympanic middle ear loss is especially perplexing because acoustic communication is dominant within Anura and tympanic middle ears enhance airborne hearing in most tetrapods. ***Methods:*** Here, we use phylogenetic comparative methods to examine whether particular geographic ranges, microhabitats, activity patterns, or aspects of acoustic communication are associated with anuran tympanic middle ear loss. ***Results:*** Although we find some differences between the geographic ranges of eared and earless species on average, there is plenty of overlap between the geographic distributions of eared and earless species. Additionally, we find a higher prevalence of diurnality in earless species, but not all earless species are diurnal. We find no universal adaptive explanation for the many instances of anuran tympanic middle ear loss. ***Conclusion:*** The puzzling lack of universally shared selection pressures among earless species motivates discussion of alternative hypotheses, including genetic or developmental constraints, and the possibility that tympanic middle ear loss is maladaptive.

Introduction

Repeated trait losses provide some of the most clear-cut examples of convergent evolution under shared selection pressures. Examples include limb loss or reduction in aquatic or fossorial mammals and squamates [1–3], loss of pigmentation and eye reduction in cavefish [4–6], and loss of the vascular cambium in aquatic plants [7]. However, trait loss research has also revealed important case studies for genetic or developmental constraint [8–13]. For example, genetic trade-offs between taste bud and eye development in cavefish may contribute to convergent eye reduction and loss [14]. Although extrinsic environmental selection pressures are often first considered in cases of convergent trait loss and are dominant in the literature, intrinsic constraints may play an equally important role in cases of convergent trait loss.

Tympanic middle ear loss within Anura (frogs and toads) provides a particularly intriguing case study to

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examine how extrinsic and intrinsic factors contribute to trait loss. The tympanic middle ear facilitates hearing on land for the vast majority of tetrapods [15], yet shows surprising evolutionary lability within Anura. The tympanic middle ear has been completely lost in anurans at least 38 different times [16], despite negative hearing consequences at higher frequencies (≥900 Hz) [17, but see 18]. Given that acoustic communication evolved early in Anura and is employed by the vast majority of anuran species [19], the prevalence of anuran tympanic middle ear loss is perplexing and we lack an adaptive explanation that broadly explains the losses.

Although tympanic middle ear loss is most prevalent in anurans, tympanic middle ear reduction and modification is associated with species habitat use in numerous other tetrapod clades. Changes to middle ear morphology are known to have occurred in burrowing mammals [20], burrowing lizards [21, 22], aquatic mammals [15, 23], and aquatic birds [24]. Anurans range in microhabitats, including aquatic, torrential, and fossorial microhabitats that could similarly alter selection on acoustic communication (e.g., high background noise in torrential habitats) and/or tympanic middle ear structures (e.g., risk of structural damage to tympanic membrane when burrowing). Additionally, changes in circadian activity patterns, such as increased diurnality, could select for visual or other communication modes that relax selection on acoustic communication. Beyond habitat use, increased frequency of tympanic membrane absence has been associated with environmental variables, such as higher elevation in the frog genus *Phrynopus* [25], indicating more macro-level habitat variables may also influence tympanic middle ear evolution. Macrohabitat variables, such as elevational range, may impose selection pressures on communication modalities and strategies or affect population sizes, influencing the likelihood of genetic drift. Although extrinsic factors provide an adaptive explanation for many convergent trait losses, it remains untested whether particular macro- or microhabitats are associated with convergent middle ear loss in anurans.

Beyond the potential for extrinsic factors to influence anuran ear loss, shared intrinsic factors among earless frogs, specifically sensory constraints related to smaller body size, could provide an explanation for anuran tympanic middle ear loss. Sensory system consequences of miniaturization have been linked to functional constraints in the salamander visual system [26] as well as the frog vestibular system [27]. Prior work has noted a relationship between smaller anuran body size and tympanic middle ear loss [28]. Frogs with smaller body sizes have less sensitive hearing, especially at higher frequencies [29] at which the tympanic middle ear enhances hearing sensitivity [17]. Frogs with smaller body sizes tend to produce higher frequency calls, and this negative relationship between body size and dominant call frequency holds across the anuran clade with very few exceptions [30]. This apparent conflict suggests that small frogs with poor high frequency hearing could experience relaxed selection on hearing conspecific calls. Alternatively, earless frogs may be avoiding hearing costs associated with tympanic middle ear loss by producing lower frequency calls, which may be sensed with non-tympanic hearing mechanisms [17, 31–33]. Yet, no study to-date has tested whether tympanic middle ear loss is more common in smaller sized anurans or compared call features of eared and earless species while accounting for phylogenetic relationships.

Here, we test whether particular environmental, ecological, or life-history traits are associated with anuran tympanic middle ear loss and can provide extrinsic or intrinsic explanations for this perplexing repeated trait loss. We compare the geographic ranges, microhabitats, and circadian activity periods of eared and earless species to determine whether ear loss is associated with particular macro- or microhabitat features. We compare body size and call dominant frequency between eared and earless species to test whether communication constraints from small body sizes and high frequency calls likely altered selection pressures on the tympanic middle ear. Examining body size and call dominant frequencies also allows us to test an alternative hypothesis that earless frogs lowered their call frequencies to escape the hearing consequences of ear loss. We find very few consistent differences between eared and earless species and we discuss remaining explanations for this widespread and puzzling trait loss.

Materials and Methods

Data Collection

Species-level data were gathered from published datasets for tympanic middle ear presence/absence [16], geographic range [34], microhabitat [35], circadian activity period [36], maximum body size [37], and call dominant frequency [30]. Species' range measurements (latitudinal degrees, elevation in meters above sea level), maximum body size (snout-vent length (mm), regardless of sex), and call dominant frequency (Hertz) were all quantified and analyzed as continuous variables. Tympanic middle ear presence/

absence was considered a binary state: eared – tympanic membrane or tympanic annulus present; earless – columella absent, which indicates all other middle ear structures (i.e., tympanic membrane, tympanic annulus, and middle ear cavity) are likewise missing. We conservatively removed species with a columella present but without a confirmed tympanic membrane or tympanic annulus because cryptic tympanic membranes and tympanic annuli can be difficult to identify externally in some species. Microhabitat included eight categories defined by Moen and Wiens [35]: (a) aquatic – almost always in water, (b) arboreal – typically on aboveground vegetation, (c) burrowing – nonbreeding season spent underground or in burrows they have dug, (d) semiaquatic – partially aquatic and partially terrestrial, (e) semiarboreal – partially arboreal and partially terrestrial, (f) semi-burrowing – partially burrowing and partially terrestrial, (g) terrestrial – found on the ground, under rocks, or in leaf litter, and (h) torrential – found in high-gradient, fast flowing streams, usually on rocks in the stream or under waterfalls. Activity diel periods were defined by Oliveira et al. [36] and included: diurnal – active during the day, nocturnal – active during the night, and crepuscular – active during the period immediately after dawn and immediately before dusk, with species categorized as active for one, two, or all three periods. All data are accessible via Dryad (10.5061/dryad.j9kd51cgd) and GitHub (https://github.com/mcwomack/BBE_EarlessEcology).

Phylogenetic Comparative Analysis

We performed all phylogenetic comparative analyses within R [38]. All analyses used an existing phylogeny from Portik et al. [39]: a concatenated maximum likelihood tree that was time-calibrated using penalized likelihood. The phylogeny was trimmed to the species within each dataset using the R packages *phytools* v1.2 [40] and *geiger* v 2.0.10 [41, 42].

We ran phylogenetic generalized least squares (PGLS) models in *caper* v. 1.0.1 [43] to test for associations between the dependent variable of tympanic middle ear presence/absence (1/0) and each of the following independent variables in turn: measures of latitudinal and elevational species' ranges (minimum, mean, maximum, and range), microhabitat, circadian activity, body size, call dominant frequency, and the interaction between body size and call dominant frequency. Models estimated phylogenetic signal (lambda) in the residual error simultaneously with the regression parameters [44]. We checked for heteroscedasticity in the PGLS models by plotting the residuals of the models and running tests to ensure models fit the assumptions. To decrease observed heteroscedasticity in the PGLS models, we log-transformed body size, call dominant frequency, and latitudinal range data and square root-transformed species elevational minimum, maximum, mean, and range data before analysis. For microhabitat, we ran two different models, one with semi-microhabitat states included as separate microhabitat categories in the model (semiarboreal, semiaquatic, etc.) and a second model with semi-microhabitat species removed from the dataset. For circadian activity patterns, we tested whether tympanic middle ear loss was associated with diurnality. For those models, we removed species that were both diurnal and nocturnal, and we also removed the small portion of species that were exclusively crepuscular (<2% of the dataset). We used an ANOVA model comparison within the *caper* package to determine if the interaction between body size and call dominant frequency explained any variation in ear presence/absence that was not already explained by body size or call dominant frequency alone. All R code and phylogenies used for analyses are accessible via Dryad (10.5061/dryad.j9kd51cgd) and GitHub (https://github.com/mcwomack/BBE_EarlessEcology).

Results

Earless frogs are geographically widespread and found at similar elevations and latitudes when compared to eared species. We found that earless species are wide-ranging geographically and are not restricted to particular areas or ecoregions. Of the 211 earless species examined here, at least one species is present on all six continents where anurans are found today (Fig. 1a).

Using a subset of species that were within the phylogeny used for our analyses and had latitudinal and elevational data (69 earless and 1,020 eared), we found that eared and earless species have similar minimum latitudes (Table 1; Fig. 1b), but earless species had lower mean and maximum latitudes and smaller latitudinal ranges compared to eared species (Table 1; Fig. 1c–e). When considering all species for which we have latitudinal and elevational data, regardless of phylogenetic information (86 earless and 1,038 eared), fewer than 10% of earless species have ranges that extend beyond 15 degrees north of the equator, and only two species, *Ascaphus truei* and *Bombina bombina*, have species ranges that extend beyond 30 degrees north of the equator. A greater percentage (33%) of earless species have ranges that extend below 15 degrees south of the equator, including 17 species with ranges that extend below 30 degrees south of the equator. Furthermore, 87% of earless species had latitudinal ranges smaller than five degrees, compared to 60% of eared species.

When comparing the elevational ranges of eared and earless species, we found earless species had higher minimum and mean elevational ranges, as well as smaller elevational ranges when compared to eared species (Table 1; Fig. 1f–g,i). However, eared and earless species had similar maximum elevational ranges (Table 1; Fig. 1h). The average earless species had a minimum elevation of 1,289 m and maximum elevation of 1,975 m, while the average eared species had a minimum elevation of 445 m and a maximum elevation of 1,436 m.

Earless Frogs Range in Microhabitat and Activity Time but Are More Likely to Be Diurnal

Our dataset includes microhabitat data for 123 earless and 1,299 eared species. Eared and earless species are found in similar proportions in aquatic (3% earless, 3%

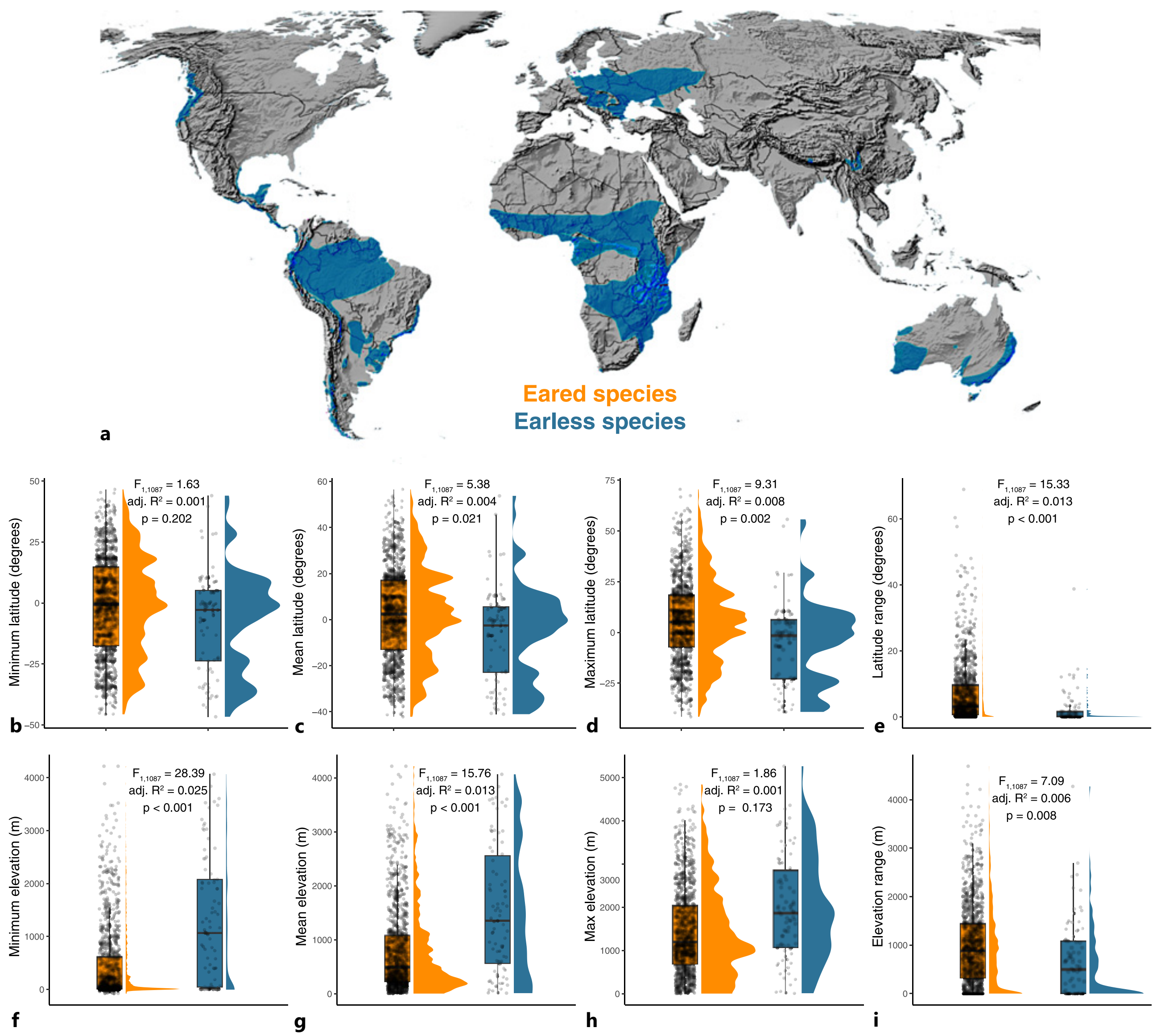

Fig. 1. Geographic distribution of earless anuran species. **a** We plotted range maps for amphibians without ears from two sources: IUCN [45] and AmphibiaWeb [46], which are both projects that create GIS polygons to represent species ranges. Earless species ranges were visualized in Quantum GIS (v 3.22.5) using the "multiply" Blending mode so that more saturated areas on the map signify overlapping ranges. Additionally, we show box plots of the latitudinal minimum (**b**), mean (**c**), maximum (**d**), and range (**e**) as well as the elevational minimum (**f**), mean (**g**), maximum (**h**), and range (**i**) of each eared (blue) and earless (orange) species' range. Dots represent individual species and half-eye plots are alongside each box plot to better visualize sampling density. Results from PGLS models are provided within panels (**b–i**).

eared), semiaquatic (5% earless, 7% eared), semiarboreal (9% earless, 9% eared), burrowing (4% earless, 3% eared), semi-burrowing (2% earless, 2% eared), and torrential microhabitats (2% earless, 4% eared). The largest microhabitat proportion differences between eared and earless species are found in terrestrial (65% earless, 37% eared) and arboreal species (10% earless, 35% eared). However, when considering species within our phylogeny (100 earless and 1,275 eared), we found no association between lacking tympanic middle ears and microhabitat

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Table 1. Results from PGLS models testing associations between tympanic middle ear presence/absence and numerous variables, with significant associations bolded and asterisks denoting significance level * <0.05, ** <0.001, *** <0.001

Variable	*N* (species)	Lambda	F statistic	Adjusted R^2	*p* value
Latitude (min)	Earless – 69 eared – 1,020	$\lambda = 0.95$	$F_{1,1087} = 1.63$	adj. $R^2 = 0.001$	$p = 0.202$
Latitude (mean)		$\lambda = 0.95$	$F_{1,1087} = 5.38$	adj. $R^2 = 0.004$	$p = 0.021$*
Latitude (max)		$\lambda = 0.95$	$F_{1,1087} = 9.31$	adj. $R^2 = 0.008$	$p = 0.002$**
Latitude (range)		$\lambda = 0.95$	$F_{1,1087} = 15.33$	adj. $R^2 = 0.013$	$p < 0.001$***
Elevation (min)		$\lambda = 0.95$	$F_{1,1087} = 28.39$	adj. $R^2 = 0.025$	$p < 0.001$***
Elevation (mean)		$\lambda = 0.95$	$F_{1,1087} = 15.76$	adj. $R^2 = 0.013$	$p < 0.001$***
Elevation (max)		$\lambda = 0.95$	$F_{1,1087} = 1.86$	adj. $R^2 = 0.001$	$p = 0.173$
Elevation (range)		$\lambda = 0.95$	$F_{1,1087} = 7.09$	adj. $R^2 = 0.006$	$p = 0.008$**
Microhabitat (with semi-microhabitats)	Earless – 100 eared – 1,275	$\lambda = 0.97$	$F_{7,1367} = 1.80$	adj. $R^2 = 0.004$	$p = 0.083$
Microhabitat (no semi-microhabitats)	Earless – 86 eared – 1,045	$\lambda = 0.97$	$F_{4,1126} = 1.66$	adj. $R^2 = 0.002$	$p = 0.157$
Circadian activity time	Earless – 42 eared – 660	$\lambda = 0.96$	$F_{1,700} = 8.46$	adj. $R^2 = 0.011$	$p = 0.004$**
Body size	Earless – 89 eared – 1,524	$\lambda = 0.95$	$F_{1,1611} = 18.55$	adj. $R^2 = 0.011$	$p < 0.001$***
Call dominant frequency	Earless – 40 eared – 909	$\lambda = 0.99$	$F_{1,947} = 0.83$	adj. $R^2 = 0.000$	$p = 0.364$
Body size*call dominant frequency	Earless – 26 Eared – 839	$\lambda = 0.99$	$F_{1,861} = 0.07$	ANOVA Model comparison	$p = 0.799$

(Fig. 2a), regardless of whether semi-microhabitats (e.g., semiaquatic) were considered as independent categories or removed from the dataset (Table 1).

Our dataset also included circadian activity patterns for 42 earless and 660 eared species within our phylogeny (Fig. 2b). We found lacking tympanic middle ears was associated with differences in circadian activity patterns (Table 1). Specifically, we found species lacking tympanic middle ears were more likely to be exclusively active during the day (diurnal) or active during the day and active during the period immediately after dawn and immediately before dusk (diurnal/crepuscular). When considering all species for which we have activity data, regardless of phylogenetic information (86 earless and 673 eared), eighty-four percent of earless species are diurnal (Fig. 2b). Meanwhile, only 25% of eared species show only diurnal or diurnal and crepuscular activity (Fig. 2b).

On Average Earless Frogs Are Slightly Smaller but Do Not Differ in Call Dominant Frequency

Using body size data from 89 earless and 1,524 eared species within our phylogeny, we found earless species have smaller body sizes, measured as maximum snout-vent length, compared to eared species (Table 1; Fig. 3a). The smallest earless species, *Brachycephalus didactylus*, had a maximum snout-vent length of 10.7 mm, which was similar in size to the smallest eared species, *Eleutherodactylus iberia* at 11 mm. However, the largest earless species, *Bufo aspinius*, was only 95 mm, while the largest eared species, *Calyptocephalella gayi* and *Conraua goliath*, had maximum snout-vent lengths of 320 mm.

Using call dominant frequency data from 40 earless and 909 eared species within our phylogeny, we found the dominant frequencies of earless species do not differ from those of eared species (Table 1; Fig. 3b). The dominant call frequency of earless species ranged from 500 to 6,800 Hz, with only three earless species, *Alsodes tumultuous, Bombina bombina, and Rhinophrynus dorsalis*, having call dominant frequencies below 1 kHz. Furthermore, the relationship between body size and dominant call frequency did not differ between eared and earless species (Table 1; Fig. 3c), indicating that earless species do not have unexpected call dominant frequencies for their body size.

Discussion

Repeated evolutionary trait losses often coincide with shared extrinsic or intrinsic factors that contribute to shared selection pressures among taxa that have convergently lost a trait. For the first time, we test whether tympanic middle ear loss in frogs is associated with particular environments, ecologies, or communication traits across a wide range of eared and earless species while accounting for phylogenetic relationships. Although we find some average differences in

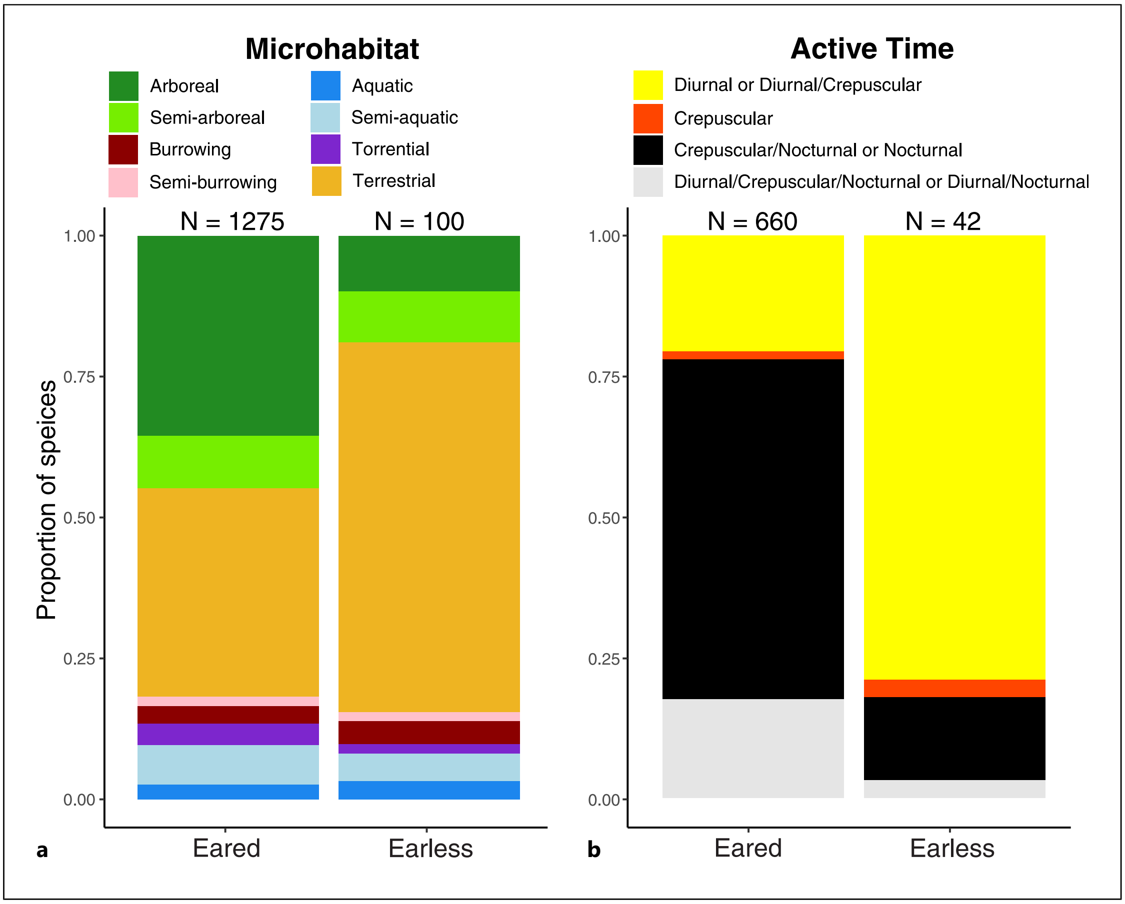

Fig. 2. a, **b** Relationship between tympanic middle ear presence/absence, microhabitat, and circadian activity periods. Proportion plots display the relative percentage of species within each microhabitat and circadian activity category. Only eared and earless species with known microhabitat or circadian activity data are displayed and the total number of eared and earless species represented in each bar is displayed at the top.

species latitudinal and elevational ranges, body size, and diurnality between eared and earless frogs, we find no universally shared condition among earless species that provides a consistent adaptive explanation for tympanic middle ear loss. Yet, several adaptive and nonadaptive explanations remain untested for earless frogs, including tympanic middle ear costs and genetic or developmental constraints (e.g., pleiotropy). Finally, it is possible that no universal explanation for tympanic middle ear loss exists and instead frogs may have lost their middle ears for various lineage-specific reasons, each of which may or may not be adaptive. The lack of universal selection pressures warrants discussion of these alternative hypotheses and the possibility that tympanic middle ear loss may be maladaptive.

Earless Frogs Are Not Geographically Distinct from Eared Frogs but Display Average Differences in Latitudinal and Elevational Ranges

Although eared anurans are found at slightly higher latitudes, earless anurans are not found in distinct geographic areas from eared anurans. Instead, earless anurans are geographically widespread and are found in areas alongside eared frogs. Although earless anuran species ranges have lower mean and maximum latitudes, this is primarily a consequence of few earless species present far north of the equator. Eared species extend to 71 degrees north of the equator whereas earless species only reach 56 degrees north of the equator. The smaller latitudinal ranges of earless species may likewise be explained by the paucity of earless species at higher latitude, as predicted by Rapoport's rule. Rapoport's rule states

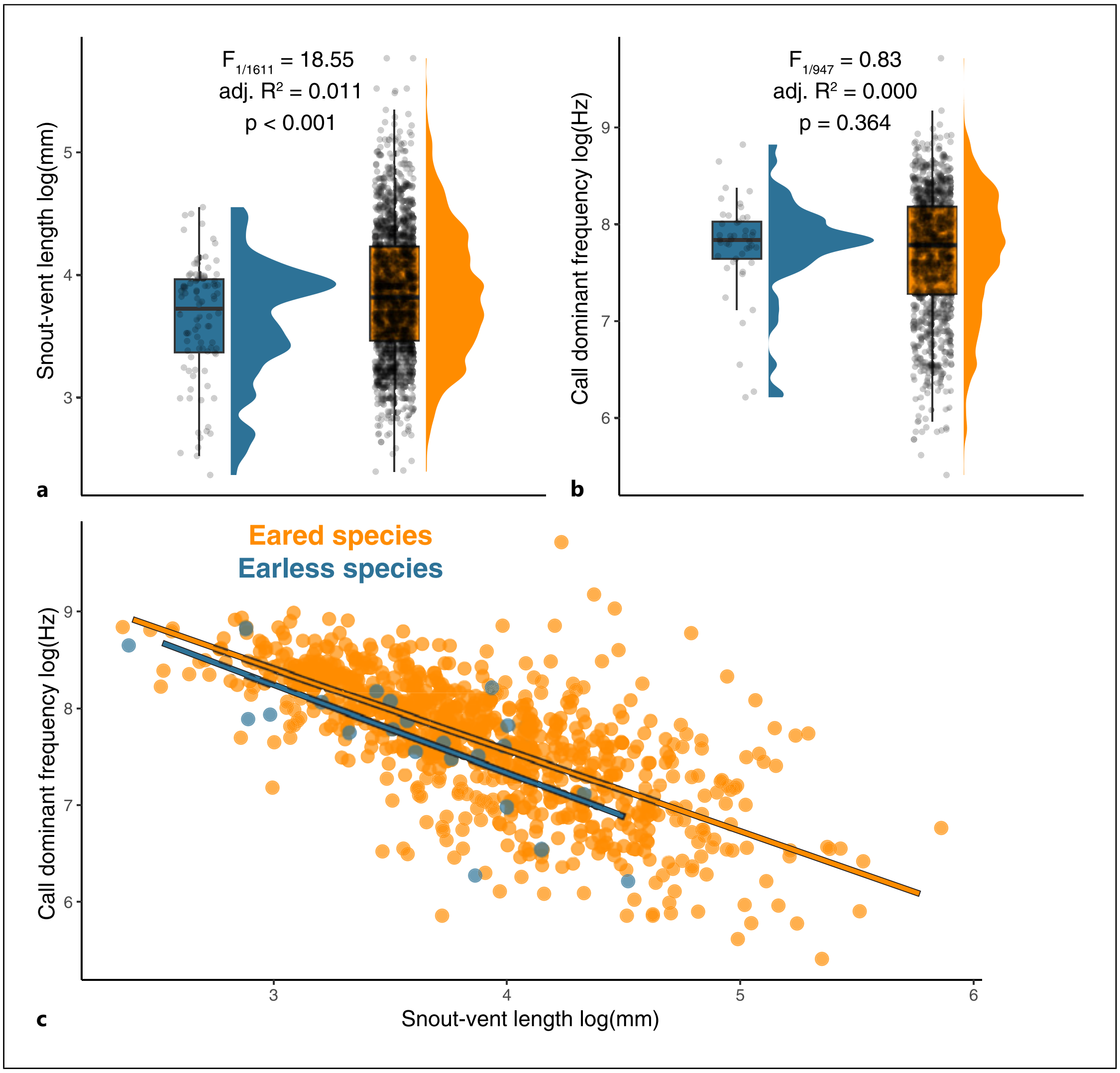

Fig. 3. Relationship between tympanic middle ear loss, body size, and dominant call frequency. Box plots compare eared (orange) and earless (blue) species snout-vent lengths (**a**) and call dominant frequencies (**b**). Additionally, we plotted the relationship between snout-vent length and call dominant frequency for species with both pieces of information available (**c**). Dots represent individual species and half eye plots are alongside each box plot to better visualize sampling density. Results from PGLS models are provided within each panel.

that range size correlates positively with distance from the equator (absolute latitude) [47] and has been supported at regional scales in amphibians [48]. However, differences in latitudinal range sizes between eared and earless species remain even when only comparing species with absolute latitudinal maximums below 25 and 15 degrees (online suppl. Fig. 1; for all online suppl. material, see https://doi.org/10.1159/000534936). Instead, it is possible the more restricted latitudinal ranges of earless species are indicative of smaller population sizes and lower genetic diversity, which support the hypothesis that drift contributes to tympanic middle ear loss. The International Union for Conservation of Nature reports that 31% of earless species are critically endangered, 20% are endangered, and an additional 10% are vulnerable, further supporting the hypothesis that earless species may have small effective population sizes [45]. However, 19% of earless species have a threat status of least concern, and tympanic middle ear loss remains puzzling as a specific and repeated consequence of drift.

Although earless anurans are found at slightly higher elevations on average, earless anurans are not found at distinct elevations from eared anurans. Earless species had higher minimum and average elevational ranges when compared to eared species. However, both eared and earless species are found from sea level up to 4,800 m above sea level. Thus, the frequency of tympanic middle ear loss increases in higher elevation species but is not restricted to high elevations. And although the elevational ranges of earless frogs appear less restricted than their latitudinal ranges, earless species had smaller elevational ranges when compared to eared species. However, when only comparing species with a latitudinal maximum below 25 or 15 degrees, there are no differences in elevational range between eared and earless species. Thus, larger elevational ranges of eared species at high latitudes seemingly driving the differences in elevational ranges between eared and earless species in the full dataset (online suppl. Fig. 2). Tympanic middle ear loss might be more common at higher elevations due to differences in the acoustic habitat or communication modalities of higher elevation species. Alternatively, higher elevation species might have smaller population sizes that facilitate drift contributing to tympanic middle ear loss. Numerous studies have found evidence of gene flow limitation among montane anuran populations [49–52], but whether there is a direct association between tympanic middle ear loss and effective population size remains untested.

Earless Frogs Share Similar Microhabitats to Eared Frogs but a Larger Proportion of Earless Species Are Diurnal

In addition to the eared and earless species overlapping in their geographic ranges, no differences in microhabitat use were found between eared and earless anurans. Given burrowing and aquatic lifestyles are associated with middle ear variation in other tetrapod clades [15, 20–24], the lack of association between anuran middle ear loss and microhabitat is surprising. There are nearly identical proportions of aquatic and burrowing earless anuran species compared to eared species. Thus, microhabitat use is not associated with middle ear evolution in anurans as in other tetrapod clades.

Increased diurnality among earless species may point toward changes in communication as a contributor to tympanic middle ear loss in some clades. The vast majority of anurans are nocturnally active (71% in this dataset) and this is reflected in the 59% of eared species which are exclusively active at night or at night and during the period immediately after dawn and immediately before dusk (nocturnal or nocturnal/crepuscular). In contrast, 80% of earless species are active during daylight (diurnal) and show no nocturnal activity. Increased predation pressure during the day could impose directional selection against loud calls and relax selection on airborne hearing. Additionally, species that are active during the day may rely disproportionately on non-acoustic communication modes, such as the visual displays of *Atelopus zeteki* [53], which might have relaxed selection on middle ears. Alternatively, tympanic middle ear loss may have evolutionarily preceded shifts to diurnality, and increased diurnality may be a response to decreased reliance on acoustic communication. Further natural history information is needed for diurnal eared and earless species to determine if and how diurnality contributes to tympanic middle ear loss.

Sensory and Communication Constraints Associated with Smaller Body Size Do Not Universally Explain Ear Loss Evolution

Although earless species are 12 mm smaller on average than eared species, the dominant frequencies of earless species' calls do not differ from eared species. Furthermore, 42 of the 45 earless species in our full dataset have a call dominant frequency greater than 900 Hz despite documented hearing consequences of tympanic middle ear loss above 900 Hz [17]. If having smaller body sizes, higher frequency calls, and poorer overall hearing relaxed selection on the tympanic middle ear, we would expect earless species to have higher frequency calls compared to eared species. Alternatively, if having lower frequency calls relaxed selection on high frequency hearing, we might expect earless species to have call dominant frequencies below 1 kHz that could be sensed by non-tympanic hearing pathways. In contrast to both alternative hypotheses, we find earless species do not have higher frequency calls than eared species and the call dominant frequencies of earless species are mostly above 1 kHz.

The commonality of earless frogs still suggests relaxed selection on airborne hearing [15], and relaxed selection on acoustic communication may be an important contributor to tympanic middle ear loss in many clades. Many earless species lack vocal sacs and lack advertisement calls entirely [25] or have weak or infrequent calls [54–56], which may reflect relaxed selection on airborne hearing in many (but not all) earless species. Although in earless clades lacking vocal sacs and/or calls, it has not been determined whether tympanic middle ears were lost prior to changes in vocal sacs or calling behavior or vice versa. Additionally, three species in our analysis had call dominant frequencies below 1 kHz, which can likely be sensed by nontympanic hearing pathways. Finally, numerous earless species with advertisement calls use additional signaling modalities, such as visual signals [53] or calling in close proximity such that substrate-borne vibrations may be more or as important than airborne hearing [57]. Thus, ear loss may only be puzzling when viewed through a lens that is focused on anuran acoustic communication. As research into other, less studied anuran sensory systems (e.g., vision – [58–61]; olfaction – [62, 63]; vibration – [64–66]) and communication styles [67] increases, tympanic middle ear loss might not seem so odd from a frog's sensory standpoint.

Caveats of Using Present-Day Data of Extant Species to Explain Trait Evolution

We should caveat our results by pointing out the limitations of testing associations between tympanic middle ear loss and present-day habitat and trait data (e.g., body size) in extant taxa. Species ranges, microhabitat use, and other traits examined here evolve over time and using present-day states could overlook key ancestral states at the time of middle ear loss. Similar issues have muddled the associations between ecology and trait loss in other clades, such as snake limb loss potentially associated with ancestral burrowing or aquatic lifestyles [68–70] and plethodontidae lung loss potentially associated with larval stream habitats [71–74]. Analyses that estimate and incorporate ancestral states (e.g., BayesTraits) when testing trait coevolution could address these issues but are unlikely to uncover a universal association between tympanic middle ear loss and particular geographic patterns, microhabitats, or circadian activity patterns. This is because tympanic middle ears are lost in many ecologically distinct clades. Therefore, we should consider alternative, intrinsic explanations for the pervasiveness of anuran tympanic middle ear loss.

Do Tympanic Middle Ears Carry Unknown Costs?

When trait losses cannot be easily rationalized, it is often assumed the lost traits have costs that would directionally select against the trait, increasing the speed and likelihood of loss in comparison to drift that would be reliant on passive mutation accumulation to generate trait loss. For example, attempts to explain eye loss in cavefish often posited that the energetic costs of developing and maintaining a functioning eye contributed to the extreme reduction in the eye when dark cave environments relaxed selection on vision [75, 76]. It is possible tympanic middle ears carry a common cost that has not been measured yet. However, earless frogs incur the energetic costs of maintaining hearing pathways and neural circuitry, as low frequency hearing via bone conduction and other nontympanic hearing pathways are preserved, and earless frogs do not noticeably differ in their inner ear structures [54, 77]. Additionally, although a number of organisms, such as parasitic trematodes, are known to parasitize the tympanic middle ear cavity and can affect hearing [78, 79], the overlapping ranges of eared and earless species do not provide confidence that increased parasite risk promotes tympanic middle ear loss. Thus, there is no current evidence to support the hypothesis that development and maintenance of the middle ear is costly and likely to be selected against, even if selection on high frequency hearing was relaxed.

Is Tympanic Middle Ear Loss the Result of Genetic or Developmental Constraint?

It is also possible that tympanic middle ears are commonly lost via pleiotropy or some other genetic or developmental constraint not yet measured. If an adaptive genetic or developmental change to one trait results in the disruption of tympanic middle ear development, then it would be selected for as long as the benefit of that genetic or developmental change outweighed any costs of tympanic middle ear loss. Pleiotropy could be the sole driver of tympanic middle ear loss or pleiotropy could trigger tympanic middle ear loss in combination with relaxed selection on high frequency hearing in certain clades. In cavefish, pleiotropic links that create a trade-off between eye development and taste bud enhancement have been implicated as a contributor to convergent eye reduction [14, but see [80]. Thus far, no evidence of a shared phenotypic change among earless species (aside from tympanic middle ear loss) has been demonstrated. Womack et al. [81] examined skull shape and presence/absence of late-forming skull features in relation to ear loss within the family Bufonidae and von May et al. [25] examined body shape difference between eared and earless species within the genus *Phrynopus*. Neither study found consistent phenotypic differences between eared and earless species. Other studies have investigated whether tympanic middle ear loss is a side effect of changes in developmental timing or rate (heterochrony), but none have found consistent shifts in development among earless species [77, 82–84]. However, these studies did not exhaustively quantify earless species phenotypes, making it possible that a shared developmental or phenotypic change exists among earless taxa. Given the nearly unlimited potential pleiotropic connections between the tympanic middle ear and other traits, genetic scans for mutations associated with tympanic middle ear loss and comparative developmental studies that examine changes during the window of arrested tympanic middle ear development [77] would allow a more thorough examination of this hypothesis.

Concluding Remarks

Earless frogs provide a captivating evolutionary puzzle that contradicts trait loss systems tied to colonization of distinct environments and shared environmental selection pressures. Tympanic middle ears appear to be repeatedly lost in the context of a variety of environmental selection pressures. It has been suggested that some cases of ear loss are "probably best viewed as an historical accident" [15], which may or may not be adaptive, while other cases of tympanic middle ear loss may be explained by some of the lineage-specific explanations we explore here (e.g., burrowing or aquatic microhabitats, low frequency calls). Regardless, the puzzling prevalence of tympanic middle ear loss suggests relaxed selection in anurans for acute hearing and provides an incentive for increased research into additional frog sensory systems and communication strategies.

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Statement of Ethics

An ethics statement was not required for this study type, no human or animal subjects or materials were used.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.C.W. gathered data, performed analyses, and wrote the manuscript. M.C.W. and K.L.H. contributed to the conceptual framework and editing.

Data Availability Statement

All data and R code are accessible via Dryad (10.5061/dryad.j9kd51cgd) and GitHub (https://github.com/mcwomack/BBE_EarlessEcology). Further inquiries can be directed to the corresponding author.

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