1 **Supplemental Information**

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3 **Echolocators are smaller than their non-echolocating relatives: agility counts in dark,**

4 **three-dimensional habitats**

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Taxon	Sub-Taxa	Echolocators (N)	Non-echolocators (N)	Estimate	SE	*Z/t*	*P*-value
All	All	1327	4878	0.454	0.065	6.984	**<0.001**
Class	Mammalia	1309	4429	0.509	0.061	8.388	**<0.001**
Order	Caprimulgiformes	18	449	0.189	0.112	1.685	**0.093**
	Chiroptera	1216	174	1.017	0.028	35.846	**<0.001**
	Rodentia	4	2258	0.752	0.287	2.625	**0.009**
	Afrosoricida	4	51	-0.664	0.248	-2.673	**0.008**
	Cetacea	76	14	1.866	0.203	9.177	**<0.001**
	Eulipotyphla	9	465	-0.031	0.085	-0.366	0.714
Family	Apodidae	17	76	0.424	0.083	5.115	**<0.001**
	Pteropodidae	8	174	0.22	0.184	1.192	0.233
	Platacanthomyidae	4	1	-	-	-	-
	Tenrecidae	4	27	-0.93	0.221	-4.202	**<0.001**
	Soricidae	5	398	-0.046	0.12	-0.381	0.703
	Talpidae	4	41	0.597	0.154	3.865	**<0.001**

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10 **Table S1. Statistical results from PGLS and GLMM models for the body mass of**

11 **echolocating vs. non-echolocating taxa.** Fixed factors in bold indicate (marginal)
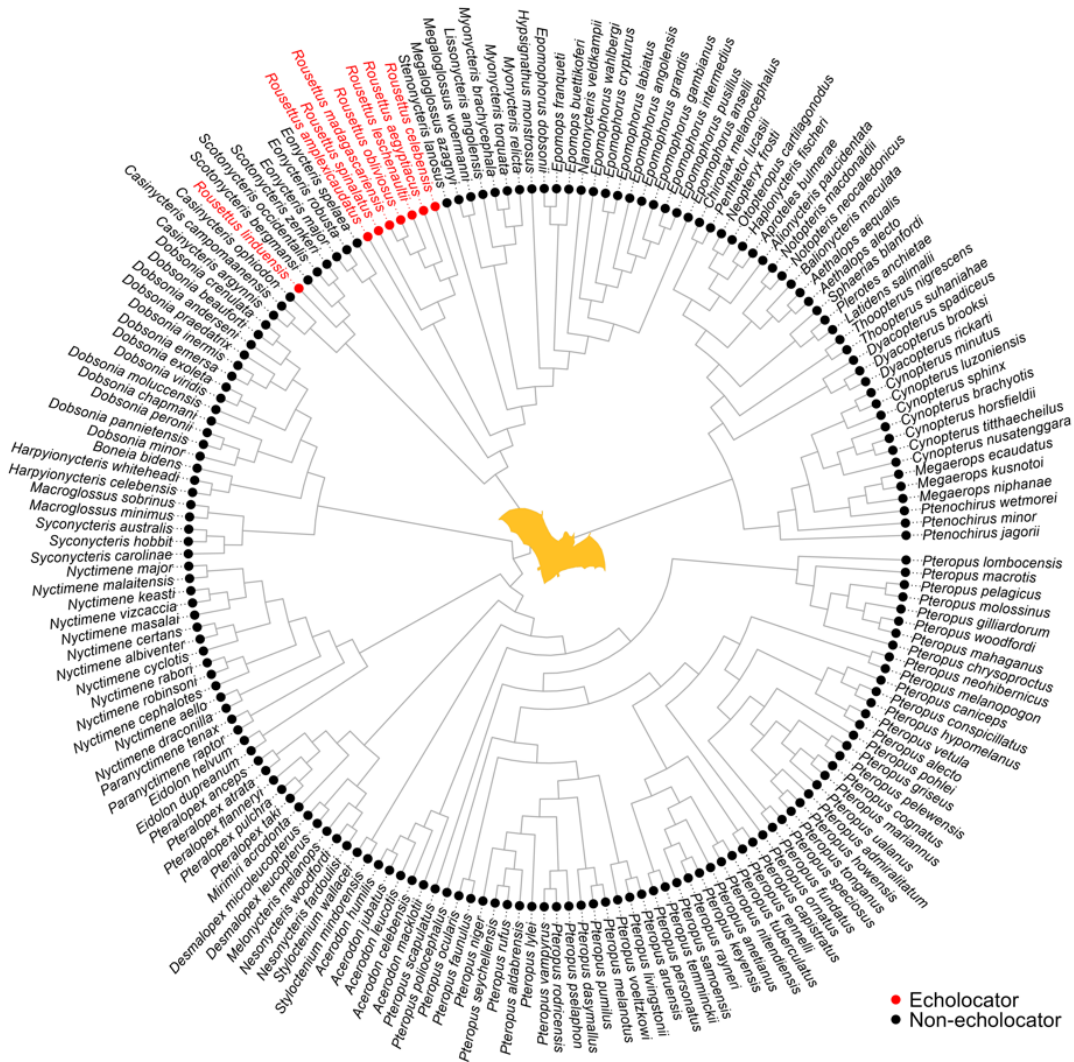
12 significant differences (P < 0.1).

Figure S1. Phylogenetic tree of echolocating and non-echolocating species in the Pteropodidae (i.e., fruit bats).

Supplemental Experimental Procedures

Collating echolocating and non-echolocating species lists

To compare body size between echolocating and non-echolocating species, we utilized the

comprehensive global list of 1,329 echolocating species (belonging six orders, Figure 1A)

provided by Wu *et al*. [S1], including Chiroptera (N = 1,218), Cetacea (N = 76), Afrosoricida (N

= 9), Eulipotyphla (N = 4), Rodentia (N = 4), and Caprimulgiformes (N = 18). The taxonomy

of non-echolocating taxa referenced from Wu *et al*. [S1] was verified against the "*Handbook of*

the Mammals of the World" [S2] (henceforth "HMW") for mammals and against the BirdLife

Taxonomic Checklist v8.1 for birds. Given that mammals accounted for the substantial

majority (98.65%) of echolocating taxa, we included all non-echolocating closely related

mammals (by class, order and family) in our comparative group to ensure appropriate

sample representation. Echolocating birds constituted only a small percentage (1.35%) of

echolocating taxa, exclusively occurring in the Caprimulgiformes; therefore, we restricted our

comparative non-echolocating group to species within this order. Because the taxonomy of

species in our list differed from phylogenetic information in the VertLife database[S3]

(vertlife.org), we matched our list of non-echolocating taxa with the VertLife phylogenetic

database (N = 4,647). For unmatched species (N = 226), we cross-referenced against

taxonomic updates from HMW[S2], and online databases (GBIF www.gbif.org; IUCN Red List,

www.iucnredlist.org; Catalogue of Life, www.catalogueoflife.org). Ultimately, we identified

phylogenetic relationships for 6,207 species, including 1,329 echolocating and 4,878

non-echolocating species.

Body mass

Body mass data were sourced from the TetrapodTraits 1.0 database[S4], which integrates

global published trait data for Tetrapod species based on the VertLife phylogenetic database.

This database employs phylogeny-informed imputation to address missing data, thereby

improving data completeness and usability for ecological and evolutionary studies, thus

allowing for better representation and analysis of global biodiversity patterns. For species

with missing body mass data (N = 44) or outliers (N = 40), we supplemented our database

47   using additional searches for body mass references in Google Scholar, HMW, and the

48   National Museum of Natural History (naturalhistory.si.edu). Only the body mass of *Eumops*

49   *chimaera* and *Carollia monohernandezi* could not be obtained. Ultimately, this body mass

50   dataset comprised 6,205 species, with all values $\log_{10}$-transformed prior to analysis.

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52   **Phylogeny**

53   To account for phylogenetic relatedness between species, we constructed a supertree using

54   available phylogenies (Loeffler-Henry et al.[S5] and Healy et al.[S6,S7]). Firstly, we generated

55   10,000 random trees for mammals and birds, based on the pseudo-posterior distributions of

56   the trimmed tree from VertLife[S3, S8]. We then calculated the ultrametric maximum clade

57   credibility (MCC) tree from these trees using the "maxCladeCred" and "chronos" functions in

58   the "phangorn" and "ape" R packages for both mammals and birds[S9, S10]. To create a

59   phylogenetic distribution encompassing both mammals and birds, we merged them into a

60   combined tree using the "tree.bind" function in the "mulTree" package[S11]. The age of the

61   amniote node was set to 315 million years, corresponding to the common ancestor of all

62   amniotes (i.e., *Archerpeton anthracos*[S5]). Ultimately, after matching species with complete

63   body mass data, our dataset included 1,327 species for echolocators, and for 4,873 species

64   for non-echolocators, and this was the sample size for our main analyses (DATA S1).

65

66   **Statistical analysis**

67   To thoroughly examine differences in body size between echolocators and related

68   non-echolocators, and considering that echolocators are distributed across different

69   phylogenetic branches within the total tree (i.e., Mammalia, Caprimulgiformes, and

70   Eulipotyphla; Figure 1A), we employed Phylogenetic Generalized Least Squares (PGLS)

71   regressions separately for the mean body mass of each species using the "phylolm" function

72   in the "phylolm" package[S12]. In parallel, because echolocators are distributed on the same

73   phylogenetic branches within their respective orders, we conducted analyses sequentially

74   for these orders and all families using Generalized Linear Mixed Models (GLMM) with the

75   "glmmTMB" function in the "glmmTMB" package[S13]. Since there was only one

76   non-echolocating species (*Platacanthomys Lasiurus*) in the Platacanthomyidae, the sample

size was too small to be included in the final model analysis. All statistical analyses were conducted using R version 4.3.0[S14].

Author Contributions

Conceptualization, J.G., N.W., Y.Z.; Methodology, J.G., N.W., J.W., Y.Z.; Data curation, J.G., N.W., J.W., J.L., H.X.; Formal analysis, J.G., N.W., Y.Z.; Visualization, J.G., N.W.; Writing – original draft, J.G., N.W.; Writing - review & editing, C.N., C.B., D.M., Y.Z.; Funding acquisition, N.W., Y.Z.; Supervision, Y.Z. All authors reviewed the results and approved the final version of the manuscript.

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