More detailed results and methods for

Echolocators are smaller than their non-echolocating relatives: agility counts in dark, three-dimensional habitats

Jinyu Guo, Nan Wu, Jie Wang, Chris Newman, Juncheng Li, Hanyu Xiang, Christina D. Buesching, David W. Macdonald, Youbing Zhou

Table S1. Statistical results from PGLS and GLMM models for the body mass of echolocating vs. non-echolocating taxa. Fixed factors in bold indicate (marginal) significant differences (P < 0.1)

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**

MATERIALS AND METHODS

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 (Figure 1, Figure S1-S5). 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 (Figure 1, Figure S6). 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

44 phylogenetic database. This database employs phylogeny-informed imputation

45 to address missing data, thereby improving data completeness and usability for

46 ecological and evolutionary studies, thus allowing for better representation and

47 analysis of global biodiversity patterns. For species with missing body mass data

48 (N = 44) or outliers (N = 40), we supplemented our database using additional

49 searches for body mass references in Google Scholar, HMW, and the National

50 Museum of Natural History (naturalhistory.si.edu). Only the body mass of

51 *Eumops chimaera* and *Carollia monohernandezi* could not be obtained.

52 Ultimately, this body mass dataset comprised 6,205 species, with all values

53 $\log_{10}$-transformed prior to analysis.

Phylogeny

55 To account for phylogenetic relatedness between species, we constructed a

56 supertree using available phylogenies (Loeffler-Henry et al.[S5] and Healy et

57 al.[S6,S7]). Firstly, we generated 10,000 random trees for mammals and birds,

58 based on the pseudo-posterior distributions of the trimmed tree from VertLife[S3,

59 S8]. We then calculated the ultrametric maximum clade credibility (MCC) tree from

60 these trees using the "maxCladeCred" and "chronos" functions in the "phangorn"

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

62 phylogenetic distribution encompassing both mammals and birds, we merged

63 them into a combined tree using the "tree.bind" function in the "mulTree"

64 package[S11]. The age of the amniote node was set to 315 million years,

65 corresponding to the common ancestor of all amniotes (i.e., *Archerpeton*

66 *anthracos*[S5]). Ultimately, after matching species with complete body mass data,

67 our dataset included 1,327 species for echolocators, and for 4,873 species for

68 non-echolocators, and this was the sample size for our main analyses (DATA

69 S1).

Statistical analysis

71 To thoroughly examine differences in body size between echolocators and

related non-echolocators, and considering that echolocators are distributed across different phylogenetic branches within the total tree (i.e., Mammalia, Caprimulgiformes, and Eulipotyphla; Figure 1A), we employed Phylogenetic Generalized Least Squares (PGLS) regressions separately for the mean body mass of each species using the "phylolm" function in the "phylolm" package[S12]. In parallel, because echolocators are distributed on the same phylogenetic branches within their respective orders, we conducted analyses sequentially for these orders and all families using Generalized Linear Mixed Models (GLMM) with the "glmmTMB" function in the "glmmTMB" package[S13]. Since there was only one 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].

Supplemental References

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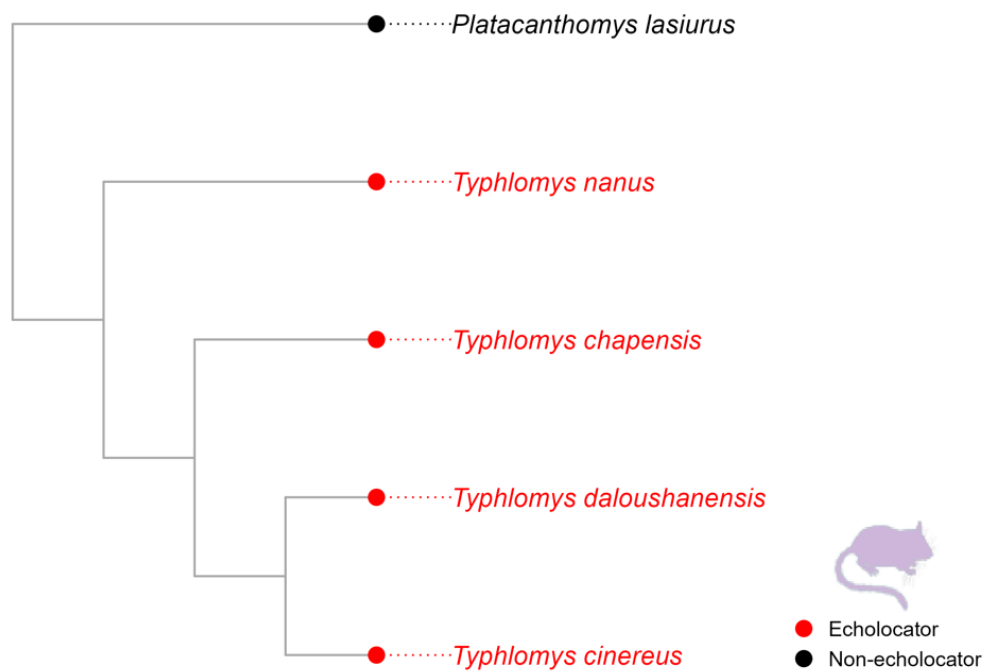119   computing (R Foundation for Statistical Computing).
120   https://www.R-project.org.

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124 **Figure S1.** Phylogenetic tree of echolocating and non-echolocating species in

125 Pteropodidae (i.e., fruit bats).

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Figure S2. Phylogenetic tree of echolocating and non-echolocating species in Platacanthomyidae.
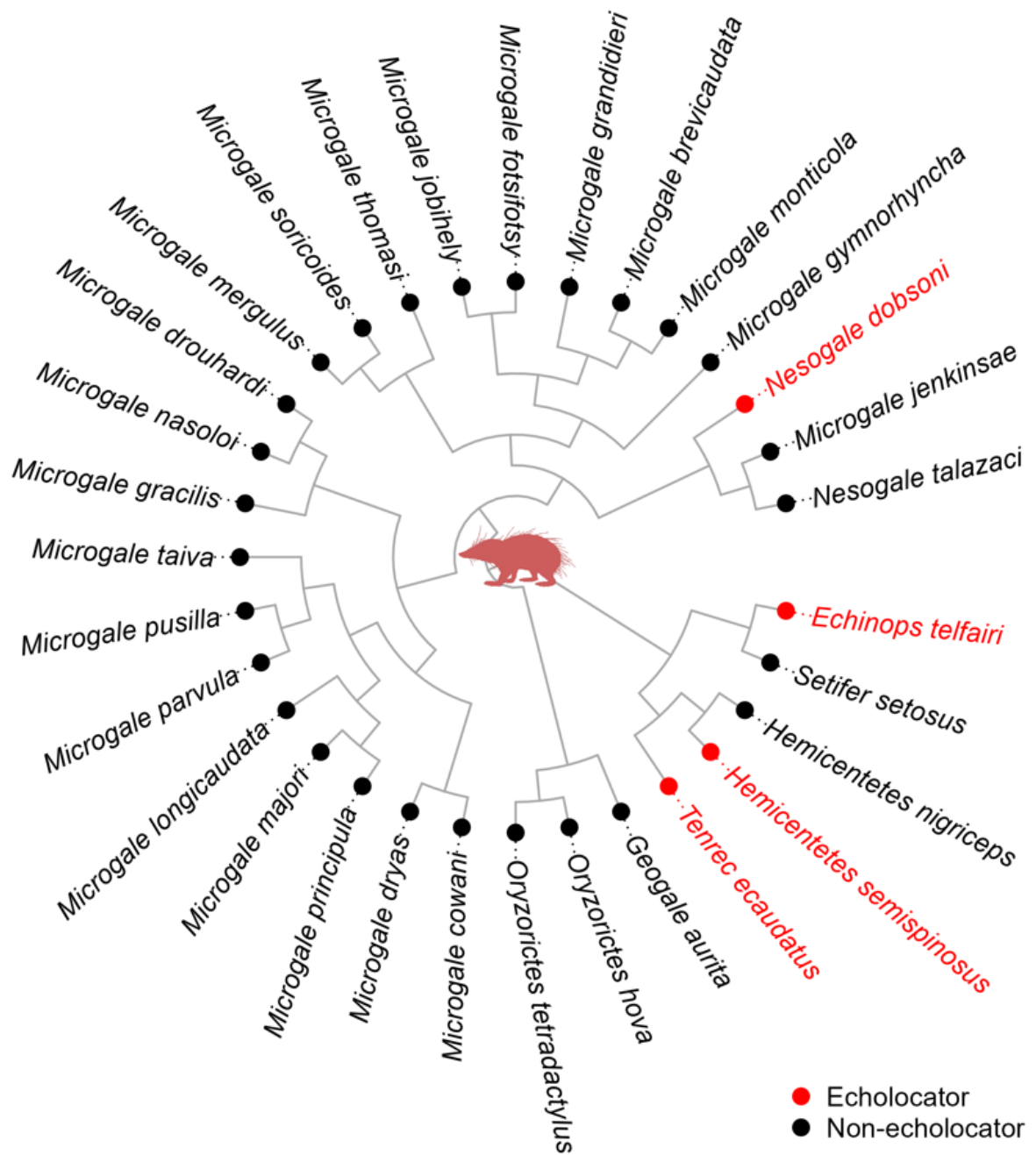
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Figure S3. Phylogenetic tree of echolocating and non-echolocating species in
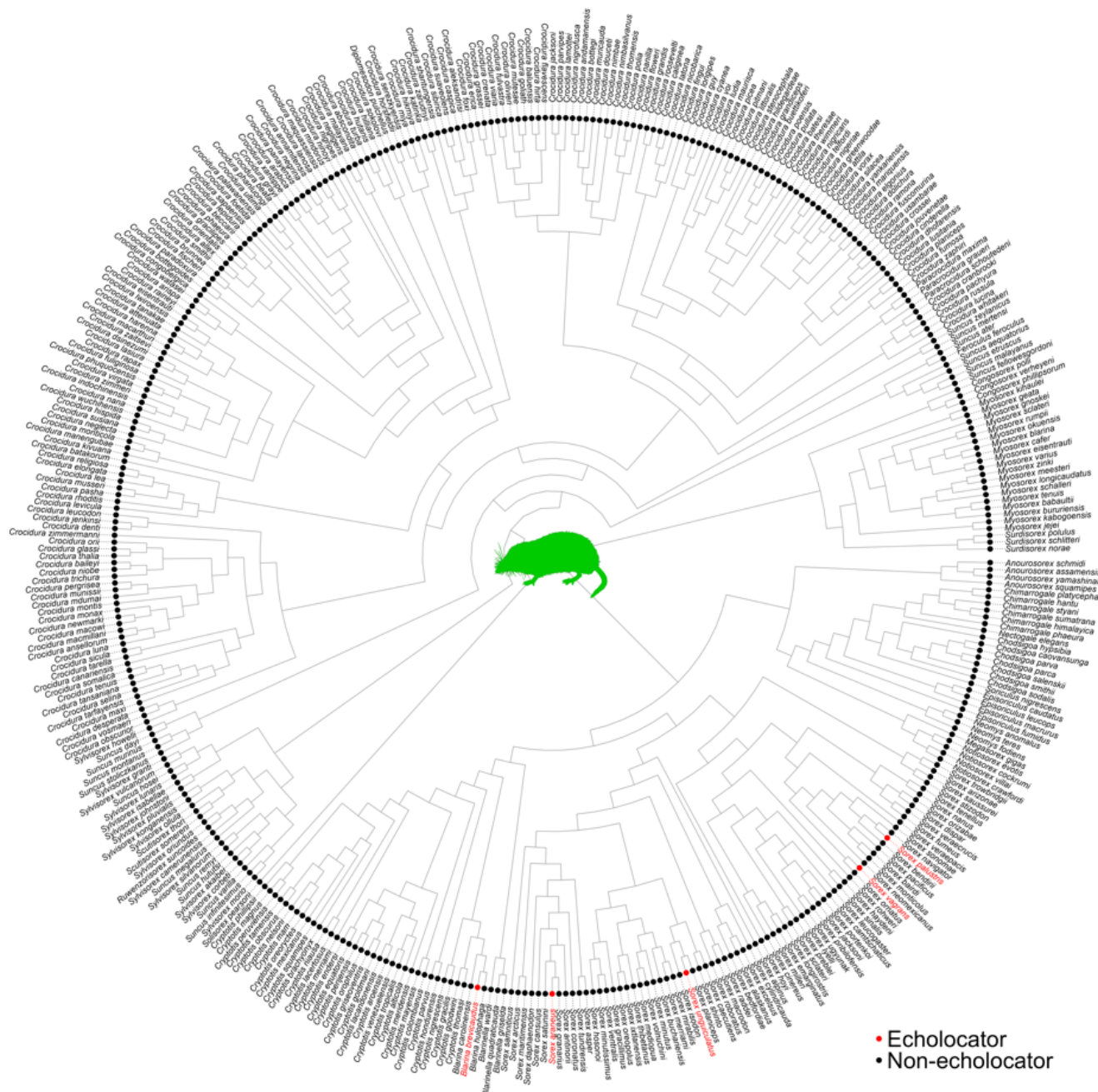
Tenrecidae.

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Figure S4. Phylogenetic tree of echolocating and non-echolocating species in
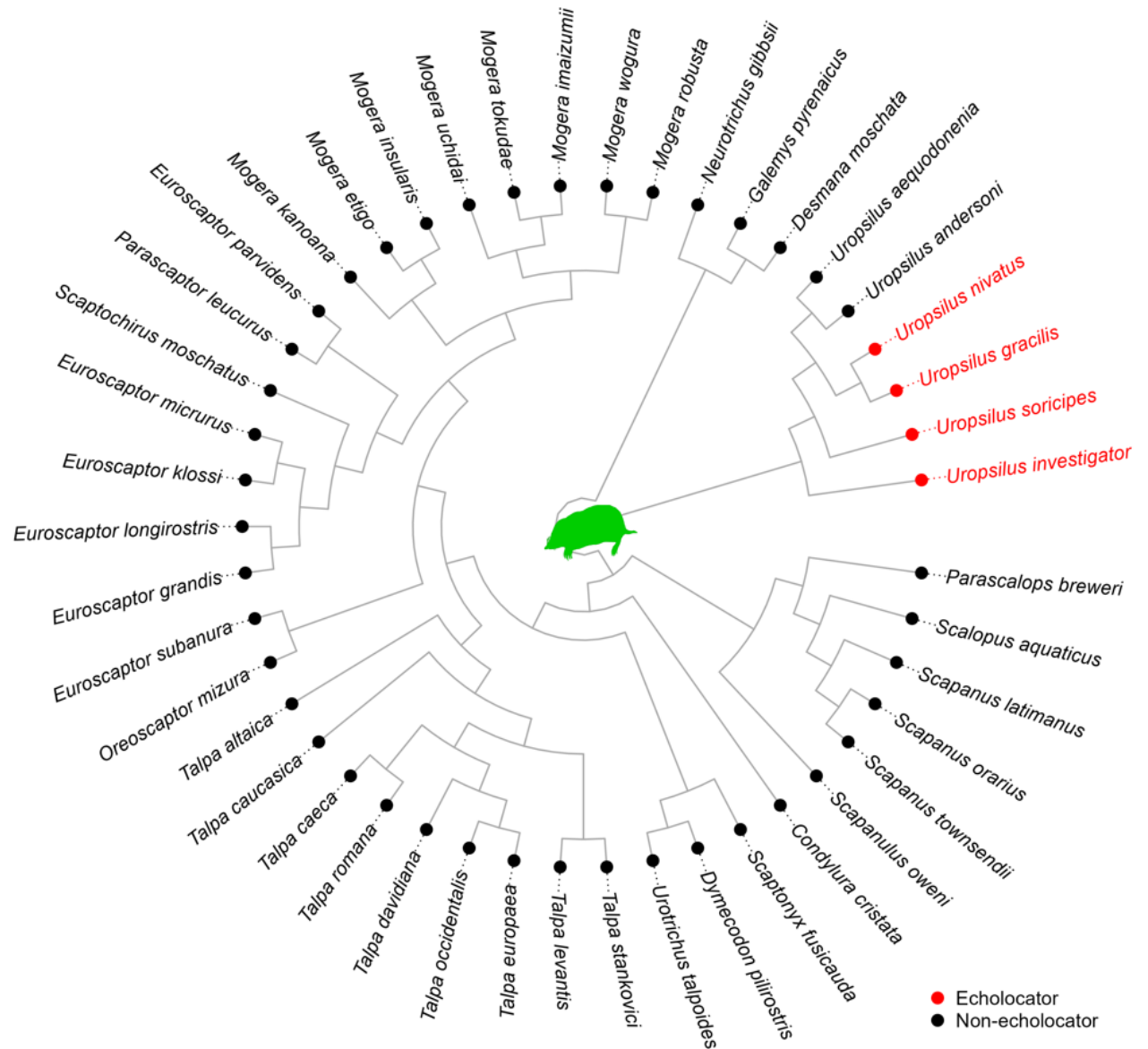
Soricidae.

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136 **Figure S5.** Phylogenetic tree of echolocating and non-echolocating species in

137 Talpidae.

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Figure S6. Phylogenetic tree of echolocating and non-echolocating species in

141 Apodidae.

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