Electronic Supplementary Materials for:

Eye size and investment in frogs and toads correlate with adult habitat, activity pattern, and breeding ecology

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Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2020.1393

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Validating specimen measurements (expanded)

Fresh specimens (n = 67) were euthanized and then immediately measured for all measurements outlined previously for museum specimens. After measuring transverse eye diameters externally, one eye was carefully dissected whole out of the specimen and transverse eye diameter measured again. Both eyes were subsequently used for other research, so remeasuring the same specimens after preservation was not possible.

We first used OLS regression to determine how externally measured ED correlates with dissected ED in fresh specimens. We then used SMA tests in smatr to determine whether preserved (museum) and fresh specimens exhibit significantly different eye-body allometric relationships, both for externally measured ED in both datasets, and for externally measured ED in preserved specimens vs. dissected. whole eye measurements in fresh specimens.

Comparison to a previous study

A recent study by Huang et al. (2019) of 44 anurans from eight families produced a dataset including SVL, mass, and CD (we back-transformed published eye volume approximations to raw CD measurements using an equation provided in that publication). We used SMA tests in smatr to compare allometric relationships between 1) CD and RM and 2) CD and SVL in that study and the present study.

This recent study also found no correlations between relative eye size (approximated from cornea diameters) and habitat type (terrestrial/arboreal vs. aquatic/semitaquatic) or activity pattern (nocturnal vs. cathemeral). Our finding of significant effects of both activity
pattern and habitat on relative eye size may result from our increased sampling and inclusion of broad ecological and taxonomic diversity. However, we also categorized adult habitat into 6 states (semiaquatic, aquatic, scansorial, ground-dwelling, subfossorial, fossorial), while the previous study used binary states (aquatic/semiaquatic vs. terrestrial/arboreal). To test whether this difference contributed to our new findings, we recategorized our species data into the binary habitat states found in Huang et al. (2019) by putting aquatic and semiaquatic species into the “aquatic/semiaquatic” category, and scansorial, ground-dwelling, fossorial, and subfossorial species into the “terrestrial/arboreal” category. We then used Kruskal-Wallis tests to test whether EDs, eye investment relative to RM, or eye investment relative to SVL differed among recategorized binary habitat states.

**Supplemental Results**

*Museum specimen measurements are reliable for eye-body allometry (expanded)*

In fresh specimens, ED measured from whole dissected eyes was highly correlated with ED measured externally prior to dissection ($R^2 = 0.96$, $n = 55$, $SE_{res} = 0.04$, $F(1,53) = 1387$, $p < 0.0001$). External ED predicted dissected ED in a log-transformed OLS regression with an allometric slope of 1.01 ($SE = 0.03$, $t = 37.2$, $p < 0.0001$) and intercept of -0.02 ($SE = 0.14$, $t = -1.11$, $p = 0.27$), indicating that external measurement slightly overestimates eye size but is a reasonable and consistent approximation (Figure S2B). AL measured from whole dissected eyes was also highly correlated with ED measured externally prior to dissection ($R^2 = 0.96$, $n = 52$, $SE_{res} = 0.04$, $F(1,50) = 1276$, $p < 0.0001$). ED predicted AL in a log-transformed OLS regression with an allometric slope of 1.02 ($SE = 0.03$, $t = 35.7$, $p < 0.0001$) and intercept of −0.06 ($SE = 0.02$, $t = -
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-2.64, p = 0.01, Figure S2A). While there is some variation in this relationship, the high correlation indicates that it is reasonable to use external transverse ED to approximate AL among anurans.

Datasets collected from fresh (externally measured and dissected ED) and preserved (externally measured ED) specimens yielded similar allometric regressions for eye scaling with body size. Allometric relationships between external ED and RM did not significantly differ in slopes (SMA test: Likelihood ratio stat. = 2.08, df = 1, p = 0.15) or intercepts (Wald stat. = 0.33, df = 1, p = 0.56) between fresh and preserved specimens (Figure S2C). Further, eye-body mass allometry derived using whole, dissected ED in fresh specimens did not show significant differences in slope (SMA test: Likelihood ratio stat. = 0.43, df = 1, p = 0.51) or intercept (Wald stat. = 2.16, df = 1, p = 0.14) from the relationship derived from externally measured ED in preserved specimens, indicating that the combination of external eye measurement and preservation introduce negligible error to estimates of anuran eye-body mass allometry (Figure S2E).

By contrast, the allometric scaling of external ED with SVL had significantly different slopes in fresh and preserved specimens (SMA test: Likelihood ratio stat. = 5.31, df = 1, p = 0.02), with a higher slope in preserved (0.94) than fresh specimens (0.83). Additionally, eye-SVL allometry derived using whole, dissected ED in fresh specimens did not show a significant difference in slope (SMA test: Likelihood ratio stat. = 2.40, df = 1, p = 0.12) from the relationship derived from externally measured ED in preserved specimens, but did show a significant difference in intercept (Wald stat. = 7.18, df = 1, p = 0.007), with a slightly higher intercept in preserved (-0.79) than in fresh specimens (-0.81, Figure S2F). Together, these results indicate
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that that ED:SVL in preserved specimens may yield slight overestimates of relative eye size compared to ED:RM, perhaps due to shrinkage in preserved specimens. However, these differences may also result from differences in species sampling across preserved and fresh specimen datasets. Regardless of cause, the differences in allometric estimates are small enough in magnitude that SVL appears to be a reasonable measure for anuran eye-body allometry if mass data are not available.

Eye-body allometry is consistent with a previous study

SMA tests comparing the scaling of CD with RM in our museum specimen data with data published by Huang et al. (2019) showed no difference in SMA slopes (Likelihood ratio stat.: 2.52, df = 3, p = 0.47) but a difference in intercepts (Wald stat.: 46.8, df = 3, p < 0.0001); the same pattern was observed for SVL (Figure S10). In both comparisons, the previously published data showed a slightly lower intercept; however, this was not a result of preservation or sampling bias at the family level, because SMA lines fitted to our own data from fresh specimens and to a subset of our museum data including only the families sampled in the photograph-based data set both yielded fits not significantly different from our full museum specimen dataset (Figure S10). Thus we conclude that, (1) the difference in intercepts is best explained by differences in measurement techniques (direct measurement vs. photographic measurement) and/or sample sizes and (2) our measurement protocol produced comparable results whether applied to museum or fresh specimens.

Habitat effects are masked in binary states
Recategorization of our species data into the binary habitat states used by Huang et al. (2019) replicated their finding that relative eye size does not differ across habitats (Figure S11). Absolute eye diameter significantly differed across binary habitat states (Kruskal-Wallis: $\chi^2 = 8.47$, df = 1, p = 0.004). However, relative eye investment did not differ across states whether measured against RM (Kruskal-Wallis: $\chi^2 = 0.03$, df = 1, p = 0.87) or SVL (Kruskal-Wallis: $\chi^2 = 0.91$, df = 1, p = 0.34).
**Supplemental Figures and Tables**

**Table S1.** Criteria used to assign species to states for ecological traits used in analyses.

| Trait states                        | Definition                                                                                     |
|-------------------------------------|------------------------------------------------------------------------------------------------|
| **Adult habitat**                   |                                                                                               |
| Semiaquatic                         | Strongly associated with/commonly found in water but also frequently uses land habitats       |
| Aquatic                             | Primarily found in water; rarely/never leaves water                                             |
| Scansorial                          | Primarily associated with plants; up off the ground (arboreal/shrubs/reeds)                  |
| Ground-dwelling                     | Primarily active on the ground                                                                |
| Subfossorial                         | Primarily active under leaf litter; shallow burrowers                                          |
| Fossorial                           | Primarily active in deeper burrows (not burrowing simply for aestivation/long periods of inactivity) |
| **Activity period**                 |                                                                                               |
| Diurnal                             | Primarily active in daylight above ground                                                     |
| Nocturnal                           | Primarily active at night above ground                                                        |
| Both                                | Regularly active during both day and at night or exclusively at transition (crepuscular)      |
| **Mating habitat**                  |                                                                                               |
| Lotic water                         | In or near moving (lotic) water (e.g. streams, rivers)                                        |
| Lentic water                        | In or near still (lentic) water or very slow-moving water (e.g. ponds, puddles)              |
| Plants                              | On/associated with plants                                                                      |
| Ground                              | On ground or leaf litter                                                                       |
| **Life history**                    |                                                                                               |
| Free-living larvae                  | Biphasic with a free-living larval stage (i.e. free-living tadpoles)                          |
| No free-living larvae               | No free-living tadpoles (direct development, viviparity, marsupiality)                        |
| **Larval habitat**                  |                                                                                               |
| No larvae                           | No free-living larvae                                                                          |
| Lotic water                         | Primarily active in flowing water (e.g. streams, rivers)                                     |
| Lentic water                        | Primarily active in still water (e.g. puddles, ponds, water trapped in plants)               |
| On land                             | Primarily active on the ground or in leaf litter                                               |
| Obscured                            | Primarily active within sand/mud/soil, foam nests in chambers, or caves                       |
| **Sexual dichromatism**             |                                                                                               |
| Present                             | Breeding females and males are markedly different colors/patterns (either developmental or dynamic) |
| Absent                              | Breeding females and males are similar colors/patterns                                          |
Figure S1. Allometric scaling relationships between measures of eye size and body size across anurans. Specimen measurements ($n = 640$) are plotted in gray diamonds, and species means ($n = 220$, used for regressions) in circles colored by adult habitat. Scaling of eye diameter with (A) the cube root of body mass and (B) snout-vent length (SVL) were similar to each other and to the scaling of corneal diameter with (E) the cube root of mass and (D) SVL. This is because cornea diameter was highly correlated with eye diameter (C), and SVL with the cube root of mass (D). Phylogenetic generalised least squares regressions (solid) of eye and cornea scaling with body size (A, B, C, D) were consistently lower in slope than either ordinary least squares regressions (dashed) or standardised major axis regressions (dotted).
**Table S2.** Parameter estimates for phylogenetic generalised least squares (PGLS) models of allometric relationships between measures of anuran eye size and body size. Models used logged species means of morphological measurements. ED = mean eye diameter, RM = cube root of mass, SVL = snout-vent length, CD = corneal diameter.

| Parameter | PGLS model |
|-----------|------------|
|           | ED vs. RM | ED vs. SVL | CD vs. ED | SVL vs. RM | CD vs. RM | CD vs. SVL |
| λ         | 0.96      | 0.96       | 0.40      | 0.75       | 0.88      | 0.87       |
| 95% conf. | 0.90, 0.99| 0.90, 1.00 | 0.06, 0.65| 0.53, 0.87 | 0.78, 0.94| 0.78, 0.94 |
| Residual SE | 0.11      | 0.10       | 0.03      | 0.04       | 0.10      | 0.09       |
| Adjusted R² | 0.80      | 0.84       | 0.96      | 0.96       | 0.73      | 0.78       |
| F-statistic| 861.9     | 1177       | 5266      | 4656       | 577.3     | 790.6      |
| df        | 1, 213    | 1, 218     | 1, 216    | 1, 213     | 1, 211    | 1, 216     |
| p-value   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| Slope     | 0.82      | 0.84       | 0.92      | 0.98       | 0.74      | 0.76       |
| SE        | 0.03      | 0.02       | 0.01      | 0.01       | 0.03      | 0.03       |
| t-value   | 29.4      | 34.3       | 72.6      | 68.2       | 24.0      | 28.1       |
| p-value   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| Intercept | 0.46      | -0.66      | -0.06     | 1.34       | 0.37      | -0.66      |
| SE        | 0.06      | 0.07       | 0.01      | 0.02       | 0.06      | 0.07       |
| t-value   | 7.29      | -9.72      | -3.85     | 62.7       | 6.64      | -9.89      |
| p-value   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
Table S3. Iterative removal of potential phylogenetic outliers (species with phylogenetic studentized residuals > |±3|) from phylogenetic generalised least squares (PGLS) models has little to no impact on parameter estimates for the allometric scaling relationships examined. Models were run iteratively until no phylogenetic outliers remained or 10 iterations were complete. ED = mean eye diameter, RM = cube root of mass, SVL = snout-vent length, CD = mean cornea diameter.

| Model/iteration | Phylogenetic outlier species removed (cumulative) | N  | Slope | Intercept | λ (95% conf.) |
|-----------------|--------------------------------------------------|----|-------|-----------|---------------|
| ED vs. RM       |                                                  |    |       |           |               |
| 1 none          |                                                  | 215| 0.82 | 0.46     | 0.96 (0.90, 0.99) |
| 2 Megophrys gerti, Oreobtes quixensis |       | 213| 0.82 | 0.46     | 0.96 (0.90, 0.99) |
| 3 Megophrys microstoma, Strabomantis bufoniformis |     | 211| 0.82 | 0.46     | 0.96 (0.90, 0.99) |
| 4 Megophrys brachycolos, Thoropa miliaris |      | 209| 0.82 | 0.46     | 0.96 (0.90, 0.99) |
| 5 Leptobrachella dringi, Proceratophrys boiei |     | 207| 0.82 | 0.46     | 0.96 (0.90, 0.99) |
| 6 Kalophrynus interlineatus |       | 206| 0.82 | 0.46     | 0.96 (0.90, 1.00) |
| 7 Rentapia hosii |         | 205| 0.82 | 0.46     | 0.96 (0.90, 1.00) |
| 8 Phrynoidis juxtaspera |      | 204| 0.81 | 0.46     | 0.97 (0.91, NA) |
| 9 Gastrophrynoides bormeensis, Callulina krefitti |    | 202| 0.81 | 0.46     | 0.97 (0.92, NA) |
| 10 Kalophrynus pleurastigma, Rana temporaria |    | 200| 0.81 | 0.46     | 0.97 (0.92, NA) |
| ED vs. SVL      |                                                  |    |       |           |               |
| 1 none          |                                                  | 220| 0.84 | -0.66    | 0.96 (0.90, 1.00) |
| 2 Heleophryne purcelli, Calyptocephalella gayi |     | 218| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 3 Leptobrachium chapaense, Mixophyes fasciolatus |   | 216| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 4 Limnodynastes salmini |        | 215| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 5 Batrachyla leptopus, Aglyptodactylus madagascariensis |    | 213| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 6 Batrachyla taeniata |       | 212| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 7 Telmatobius culeus |       | 211| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 8 Lepidobatrachus laevis, Microhyla fusca |     | 209| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 9 Hylodes nasus, Microhyla pulverata |      | 207| 0.84 | -0.67    | 0.96 (0.90, 1.00) |
| 10 Gastrophrynoides bormeensis |    | 206| 0.83 | -0.66    | 0.96 (0.90, 1.00) |
| ED vs. CD       |                                                  |    |       |           |               |
| 1 none          |                                                  | 218| 0.92 | -0.06    | 0.40 (0.06, 0.65) |
| 2 Spelaeophyne methneri |       | 217| 0.92 | -0.06    | 0.40 (0.07, 0.66) |
| 3 Breviceps mossambicus, Aglyptodactylus madagascariensis |  | 215| 0.92 | -0.06    | 0.39 (0.06, 0.65) |
| 4 Bombina orientalis, Aplastodiscus albofrenatus |   | 213| 0.92 | -0.06    | 0.40 (0.07, 0.65) |
| 5 Ptychadena oxyrhynchus |         | 212| 0.92 | -0.06    | 0.40 (0.07, 0.65) |
| 6 Bombina fortinuptialis, Conraua crassipes |    | 210| 0.92 | -0.06    | 0.41 (0.08, 0.66) |
| 7 Rhinophrynus dorsalis |       | 209| 0.93 | -0.06    | 0.00 (NA, 0.63) |
| SVL vs. RM      |                                                  |    |       |           |               |
| 1 none          |                                                  | 215| 0.98 | 1.34     | 0.75 (0.53, 0.87) |
| 2 Rana pipiens |           | 214| 0.98 | 1.34     | 0.75 (0.54, 0.87) |
| 3 Pipa pipa     |           | 213| 0.98 | 1.34     | 0.74 (0.53, 0.87) |
| CD vs. RM       |                                                  |    |       |           |               |
| 1 none          |                                                  | 213| 0.75 | 0.37     | 0.88 (0.78, 0.94) |
| 2 Aglyptodactylus madagascariensis |      | 212| 0.75 | 0.37     | 0.88 (0.78, 0.94) |
| 3 Breviceps mossambicus, Megophrys gerti, Breviceps mossambicus | | 209| 0.75 | 0.37     | 0.88 (0.77, 0.94) |
| 4 Alytes obstetricans, Micrixalus phylophilus |     | 207| 0.75 | 0.36     | 0.88 (0.78, 0.94) |
| 5 Discoglossus pictus, Pipa carvalhoi |       | 205| 0.77 | 0.34     | 0.94 (0.87, 0.98) |
| 6 Leioptelma hochstetteri |      | 204| 0.77 | 0.32     | 0.94 (0.86, 0.98) |
| 7 Leptodactylus melanotus |       | 203| 0.76 | 0.32     | 0.94 (0.86, 0.98) |
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|  | Species | CD vs. SVL | p-value | CI (95%) |
|---|---------|------------|---------|----------|
| 8 | Barbourula busuangensis, Incilius nebulifer | 201 | 0.78 | 0.30 | 0.96 (0.90, 0.99) |
| 9 | Leptodactylus bolivianus | 200 | 0.79 | 0.30 | 0.96 (0.89, 0.99) |
| 1 | none | 218 | 0.76 | -0.66 | 0.87 (0.78, 0.94) |
| 2 | Alytes obstetricans, Bombina orientalis, Allophryne ruthveni, Aglyptodactylus madagascariensis | 214 | 0.76 | -0.66 | 0.87 (0.77, 0.94) |
| 3 | Discoglossus pictus, Rhinophrynus dorsalis, Mantella aurantiaca | 211 | 0.76 | -0.65 | 0.86 (0.76, 0.93) |
| 4 | Leiopelma hochstetteri, Eleutherodactylus planirostris | 209 | 0.76 | -0.67 | 0.86 (0.76, 0.93) |
| 5 | Hyla cinerea | 208 | 0.77 | -0.67 | 0.86 (0.76, 0.94) |
| 6 | Eleutherodactylus coqui | 207 | 0.77 | -0.67 | 0.87 (0.76, 0.94) |
| 7 | Craugastor fitzingeri | 206 | 0.77 | -0.67 | 0.87 (0.76, 0.94) |
| 8 | Diasporus diastema | 205 | 0.77 | -0.67 | 0.87 (0.76, 0.94) |
| 9 | Craugastor podiciferus, Tricobatrachus robustus | 203 | 0.77 | -0.68 | 0.87 (0.76, 0.94) |
| 10 | Bombina fortinuptialis, Eleutherodactylus marnockii | 201 | 0.77 | -0.68 | 0.87 (0.76, 0.94) |
**Table S4.** Parameter estimates for ordinary least squares (OLS) and standardized major axis (SMA) models fit to the same logged species data for anuran eye and body size used in phylogenetic generalised least squares (PGLS) models. ED = mean eye diameter, RM = cube root of mass, SVL = snout-vent length, CD = mean cornea diameter.

| Model and outputs | Comparison       | ED vs. RM | ED vs. SVL | CD vs. ED | SVL vs. RM | CD vs. RM | CD vs. SVL |
|-------------------|------------------|-----------|------------|-----------|------------|-----------|------------|
| **OLS**           |                  |           |            |           |            |           |            |
| Residual SE       |                  | 0.12      | 0.10       | 0.03      | 0.04       | 0.12      | 0.11       |
| Adj. R²           |                  | 0.68      | 0.76       | 0.97      | 0.96       | 0.63      | 0.71       |
| F-statistic       |                  | 461.6     | 700.1      | 6789      | 4568       | 360.5     | 538.9      |
| df                |                  | 1, 213    | 1, 218     | 1, 216    | 1, 213     | 1, 211    | 1, 216     |
| p-value           |                  | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| Slope             |                  | 0.85      | 0.88       | 0.93      | 0.99       | 0.76      | 0.79       |
| SE                |                  | 0.04      | 0.03       | 0.01      | 0.01       | 0.04      | 0.03       |
| t-value           |                  | 21.5      | -13.0      | 82.4      | 67.6       | 19.0      | 23.2       |
| p-value           |                  | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| Intercept         |                  | 0.47      | -0.71      | -0.06     | 1.34       | 0.39      | -0.68      |
| SE                |                  | 0.01      | 0.05       | 0.01      | 0.01       | 0.01      | 0.06       |
| t-value           |                  | 32.5      | 26.5       | -6.80     | 246.5      | 26.2      | -12.1      |
| p-value           |                  | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| **SMA**           |                  |           |            |           |            |           |            |
| R²                |                  | 0.68      | 0.76       | 0.97      | 0.96       | 0.63      | 0.71       |
| p-value           |                  | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   | < 0.0001  | < 0.0001   |
| Slope             |                  | 0.99      | 0.96       | 0.94      | 1.02       | 0.92      | 0.87       |
| Limits            |                  | 0.93, 1.06| 0.91, 1.00 | 0.92, 0.96| 0.98, 1.04 | 0.85, 0.99| 0.82, 0.93 |
| Intercept         |                  | 0.44      | -0.82      | -0.07     | 1.33       | 0.36      | -0.80      |
| Limits            |                  | 0.42, 0.47| -0.90, -0.74| -0.09, -0.05| 1.32, 1.34 | 0.33, 0.38| -0.88, -0.71|
| Isometry          |                  | yes       | yes        | yes       | yes        | yes       | yes        |
| Test stat (r)     |                  | -0.02     | -0.14      | 0.07      |            |           |            |
| df                |                  | 213       | 218        | 213       |            |           |            |
| p-value           |                  | 0.77      | 0.06       | 0.29      |            |           |            |
Figure S2. External measurements of anuran eye size can predict dissected measurements (A-B), and eye scaling relationships derived from a large sample of preserved museum specimens yield similar fits to those derived from a smaller sample of fresh specimens (C-F). (A) Axial eye length (AL) measured from dissected eyes is highly correlated with transverse eye diameter (ED) measured externally prior to dissection in fresh specimens ($R^2 = 0.96$, $n = 52$, SE$_{res.} = 0.04$,
F(1,50) = 1276, p < 0.0001). ED predicts AL in a log-transformed ordinary least squares regression with an allometric slope of 1.02 (SE = 0.03, t = 35.7, p < 0.0001) and intercept of -0.06 (SE = 0.02, t = -2.64, p = 0.01). (B) ED measured from dissected eyes is highly correlated with ED measured externally prior to dissection in fresh specimens (R^2 = 0.96, n = 55, SE_res. = 0.04, F(1,53) = 1387, p < 0.0001). External ED predicts dissected ED in a log-transformed ordinary least squares regression with an allometric slope of 1.01 (SE = 0.03, t = 37.2, p < 0.0001) and intercept of -0.02 (SE = 0.14, t = -1.11, p = 0.27). (C) Externally measured ED scales with the cube root of mass (RM) allometrically via equal slopes (SMA test: Likelihood ratio stat. = 2.08, df = 1, p = 0.15) and equal intercepts (Wald stat. = 0.33, df = 1, p = 0.56) in fresh and preserved specimens. (D) Externally measured ED scales with snout-vent length (SVL) with unequal slopes in fresh and preserved specimens (SMA test: Likelihood ratio stat. = 5.31, df = 1, p = 0.02). The slope estimate was higher for preserved specimens (0.94) than for fresh specimens (0.83), which may indicate some shrinkage in SVL of preserved specimens. (E) Externally measured ED from preserved specimens scales with RM allometrically with the same slope (SMA test: Likelihood ratio stat. = 0.43, df = 1, p = 0.51) and intercept (Wald stat. = 2.16, df = 1, p = 0.14) as ED measured from dissected eyes of fresh specimens. This indicates that using preserved specimens and external measurements provides an accurate estimate of eye scaling in anurans. (F) Externally measured ED from preserved specimens scales with SVL allometrically with the same slope (SMA test: Likelihood ratio stat. = 2.40, df = 1, p = 0.12) but a different intercept (Wald stat. = 7.18, df = 1, p = 0.007) than measurements of ED from dissected eyes of fresh specimens. The intercept for preserved specimens was slightly higher (-0.79) than fresh specimens (-0.81), indicating that using preserved specimens may slightly overestimate relative eye sizes but is a close approximation.
Figure S3. Compiled data for vertebrate eye and body size separated by eye measurement (axial length or transverse diameter), body size measurement (mass or snout-vent length), and vertebrate clade. Allometric scaling relationships were similar for phylogenetic generalised least squares (solid) and ordinary least squares (dashed) models.
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Table S5. Phylogenetic generalised least squares (PGLS) regression fits for vertebrate eye scaling. AL = axial eye length, RM = cube root of mass, ED = transverse eye diameter, SVL = snout-vent length.

| PGLS model     | n   | λ   | 95% conf. (λ) | Res. SE | Adj. R² | F-stat. | df  | p-value | Slope (a) | SE_a | t-value_a | p-value_a | Intercept (b) | SE_b | t-value_b | p-value_b |
|----------------|-----|-----|---------------|---------|---------|---------|-----|---------|-----------|------|-----------|-----------|----------------|------|-----------|-----------|
| AL vs. RM      |     |     |               |         |         |         |     |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 215 | 0.95| 0.88, 0.99    | 0.09    | 0.80    | 877.5  | 1, 213| <0.0001 | 0.73      | 0.02 | 29.6      | <0.0001  | 0.57           | 0.05 | 10.8      | <0.0001  |
| Birds          | 139 | 0.88| 0.67, 0.96    | 0.01    | 0.37    | 82.63  | 1, 137| <0.0001 | 0.45      | 0.05 | 9.09      | <0.0001  | 0.71           | 0.08 | 9.28      | <0.0001  |
| Mammals        | 181 | 0.94| 0.89, 0.97    | 0.02    | 0.58    | 252.4  | 1, 179| <0.0001 | 0.58      | 0.04 | 15.89     | <0.0001  | 0.29           | 0.16 | 1.88      | 0.06      |
| Ray-finned fishes | 205 | 0.94| 0.87, 0.97    | 0.01    | 0.76    | 631.8  | 1, 203| <0.0001 | 0.69      | 0.03 | 25.1      | <0.0001  | 0.38           | 0.08 | 4.54      | <0.0001  |
| Sharks & rays  | 41  | 1.00| 0.37, NA      | 0.01    | 0.44    | 32.1   | 1, 39 | <0.0001 | 0.43      | 0.08 | 5.67      | <0.0001  | 0.70           | 0.11 | 6.66      | <0.0001  |
| AL vs. SVL     |     |     |               |         |         |         |     |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 220 | 0.96| 0.90, 1.00    | 0.10    | 0.84    | 1174   | 1, 218| <0.0001 | 0.85      | 0.02 | 34.3      | <0.0001  | -0.68          | 0.07 | -9.75     | <0.0001  |
| Squamate lizards | 106 | 0.63| 0.35, 0.83    | 0.01    | 0.51    | 109.7  | 1, 104| <0.0001 | 0.57      | 0.05 | 10.5      | <0.0001  | -0.38          | 0.13 | -2.91     | 0.04      |
| ED vs. SVL     |     |     |               |         |         |         |     |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 220 | 0.96| 0.90, 1.00    | 0.10    | 0.84    | 1174   | 1, 218| <0.0001 | 0.84      | 0.02 | 34.3      | <0.0001  | -0.66          | 0.07 | -9.72     | <0.0001  |
| Squamates      | 127 | 1.00| 0.79, NA      | 0.02    | 0.67    | 86.2   | 3, 123| <0.0001 | 0.86      | 0.06 | 13.5      | <0.0001  | -1.88          | 0.24 | -7.69     | <0.0001  |
| Colubrid snakes | 66  |     | 0.86          | 0.06    | 13.5    | <0.0001| -1.88 | 0.24     | -7.69     |      |           |           |                |      |           |           |
| Geckos         | 61  |     | 0.94          | 0.37    | 14.1    | 0.52   | -1.15 | 0.60     | -7.04     |      |           |           |                |      |           |           |
| ED vs. RM      |     |     |               |         |         |         |     |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 215 | 0.96| 0.90, 0.99    | 0.11    | 0.80    | 861.9  | 1, 213| <0.0001 | 0.82      | 0.03 | 29.4      | <0.0001  | 0.46           | 0.06 | 7.29      | <0.0001  |
| Teleost fishes | 299 | 0.99| 0.96, 1.00    | 0.01    | 0.63    | 510.2  | 1, 297| <0.0001 | 0.72      | 0.03 | 22.6      | <0.0001  | 0.58           | 0.06 | 10.2      | <0.0001  |
**Table S6.** Ordinary least squares (OLS) regression fits for vertebrates. AL = axial eye length, RM = cube root of mass, ED = transverse eye diameter, SVL = snout-vent length.

| OLS model       | n     | Res. SE | Adj. R² | F-stat. | df    | p-value | Slope (a) | SEₐ | t-valueₐ | p-valueₐ | Intercept (b) | SEₐ | t-valueₐ | p-valueₐ |
|-----------------|-------|---------|---------|---------|-------|---------|-----------|------|-----------|-----------|----------------|------|-----------|-----------|
| **AL vs. RM**   |       |         |         |         |       |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 215   | 0.10    | 0.69    | 485.5   | 1, 213| <0.0001 | 0.74      | 0.03 | 22.0      | <0.0001 | 0.59           | 0.01 | 47.3      | <0.0001 |
| Birds           | 139   | 0.12    | 0.65    | 260.9   | 1, 137| <0.0001 | 0.58      | 0.04 | 16.2      | <0.0001 | 0.61           | 0.03 | 18.3      | <0.0001 |
| Mammals         | 181   | 0.18    | 0.79    | 689.8   | 1, 179| <0.0001 | 0.68      | 0.03 | 26.3      | <0.0001 | 0.28           | 0.03 | 8.08      | <0.0001 |
| Ray-finned fishes | 215  | 0.15    | 0.70    | 502     | 1, 213| <0.0001 | 0.68      | 0.03 | 22.4      | <0.0001 | 0.42           | 0.02 | 23.9      | <0.0001 |
| Sharks & rays   | 41    | 0.13    | 0.60    | 60.5    | 1, 39 | <0.0001 | 0.47      | 0.06 | 7.78      | <0.0001 | 0.69           | 0.08 | 9.90      | <0.0001 |
| **AL vs. SVL**  |       |         |         |         |       |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 220   | 0.10    | 0.76    | 696.7   | 1, 218| <0.0001 | 0.89      | 0.03 | 26.4      | <0.0001 | -0.72          | 0.06 | -13.2     | <0.0001 |
| Squamate lizards | 107  | 0.14    | 0.51    | 111     | 1, 105| <0.0001 | 0.52      | 0.05 | 10.5      | <0.0001 | -0.32          | 0.10 | -3.28     | 0.001    |
| **ED vs. SVL**  |       |         |         |         |       |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 220   | 0.10    | 0.76    | 700.1   | 1, 218| <0.0001 | 0.88      | 0.03 | 26.5      | <0.0001 | -0.71          | 0.05 | -13.0     | <0.0001 |
| Squamates      | 127   | 0.11    | 0.68    | 91.1    | 3, 123| <0.0001 | 0.89      | 0.08 | 11.6      | <0.0001 | -1.93          | 0.20 | -9.47     | <0.0001 |
| Colubrid snakes | 66    | 0.89    | 0.08    | 11.6    |       | <0.0001 | 1.93      |       |           |           |                |      |           |           |
| Geckos          | 61    | 0.91    | 0.19    | 11.8    |       | <0.0001 | 1.09      |       |           |           |                |      |           |           |
| **ED vs. RM**   |       |         |         |         |       |         |           |      |           |           |                |      |           |           |
| Frogs & toads  | 215   | 0.12    | 0.68    | 461.6   | 1, 213| <0.0001 | 0.85      | 0.04 | 21.5      | <0.0001 | 0.47           | 0.01 | 32.5      | <0.0001 |
| Teleost fishes | 306   | 0.14    | 0.57    | 406.4   | 1, 304| <0.0001 | 0.77      | 0.04 | 20.2      | <0.0001 | 0.54           | 0.02 | 31.7      | <0.0001 |
Figure S4. Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size across adult habitats. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by mating habitat. Meg. = Megophryidae, Brev. = Brevicepitidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each adult habitat state. Kruskal-Wallis tests treating species as independent showed significant differences in absolute eye size, eye investment relative to mass, and eye investment relative to SVL, but not cornea investment relative to eye size among adult habitats. Phylogenetic ANCOVAs indicated that habitat has significant effects on eye size relative to mass, eye size relative to SVL, and cornea size relative to eye size.
Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)
**Figure S5.** Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size across mating habitats. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by mating habitat. Meg. = Megophryidae, Brev. = Brevicipitidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each mating habitat state. Kruskal-Wallis tests treating species as independent showed significant differences in absolute eye size, eye investment relative to mass, eye investment relative to SVL, and cornea investment relative to eye size among mating habitats. Phylogenetic ANCOVAs indicated that mating habitat has significant effects on eye size relative to mass, eye size relative to SVL, and cornea size relative to eye size.
**Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)**

**A**

| Family            | Pipidae | Meg. | Microhylidae | Brev. | Arthro. | Pyx. | Dicro. | Ranidae | Myo. | Hem. | Hylidae | Ele. | Lep. | Bufonidae |
|-------------------|---------|------|--------------|-------|---------|------|--------|---------|------|------|---------|-----|------|-----------|
| Eye size (mm)     | 0-8     | 8-12 | 1.6x         | 1.25x | 0.25x   | 1.0x | 0.63x  | 1.0x    | 0.63x| 0.40x| 0.40x   | 0.25x| 0.40x| 0.79x     |
| Investment        | Absent  | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Present | Present | Present |

**B**

| Sex dichromatism | Sex dichromatism | Sex dichromatism | Sex dichromatism |
|------------------|------------------|------------------|------------------|
| Mean eye diameter (mm) | Eye investment (mass) | Eye investment (SVL) | Corneal investment |

**C, D, E**

| Sex dichromatism | Sex dichromatism | Sex dichromatism |
|------------------|------------------|------------------|
| 1.6x             | 1.0x             | 1.0x             |
| 1.0x             | 0.63x            | 0.79x            |
| 0.63x            | 0.40x            | 1.0x             |
| 0.40x            | 0.25x            | 0.40x            |
| 0.25x            |                  |                  |

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Figure S6. Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size across sexual dichromatism states. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and phylogenetically corrected eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by the presence or absence of sexual dichromatism. Meg. = Megophryidae, Brev. = Brevicophiidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each sexual dichromatism state. Kruskal-Wallis tests treating species as independent showed no significant differences in absolute eye size, eye investment relative to mass, eye investment relative to SVL, or cornea investment relative to eye size among sexual dichromatism states. Phylogenetic ANCOVAs indicated that sexual dichromatism has no significant effects on eye size relative to mass, eye size relative to SVL, or cornea size relative to eye size.
Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)

Eye size (mm)

A

Pipidae  Meg.
Microhylidae  Brev.
Arth.  Pyx.
Dicro.  Ranidae  Myo.
Hem.  Hylidae  Ele.
Lep.  Bufonidae

Investment

B

C

D

E

Eye diameter (mm)

Activity period

Eye investment (mass)

Activity period

Eye investment (S/L)

Activity period

Corneal investment

Activity period
Figure S7. Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size across adult activity patterns. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by activity pattern. Meg. = Megophryidae, Brev. = Brevicepitidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each activity pattern state. Kruskal-Wallis tests treating species as independent showed significant differences in absolute eye size, but no significant differences in eye investment relative to mass, eye investment relative to SVL, or cornea investment relative to eye size among activity patterns. Phylogenetic ANCOVAs indicated that activity pattern has significant effects on eye size relative to mass, eye size relative to SVL, and cornea size relative to eye size.
Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)

A

B

C

D

E

Free–living larvae
No free–living larvae
**Figure S8.** Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size in anurans with and without free-living larvae. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by life history strategy. Meg. = Megophryidae, Brev. = Brevicepitidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each life history state. Kruskal-Wallis tests treating species as independent showed significant differences in absolute eye size and cornea investment relative to eye size, but no significant difference in eye investment relative to mass or eye investment relative to SVL among life history strategies. Phylogenetic ANCOVAs indicated that life history has no significant effects on eye size relative to mass, eye size relative to SVL, or cornea size relative to eye size.
**Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)**

| Family     | Microhylidae | Brev. | Arthro. | Pyx. | Dicro. | Ranidae | Myo. | Hem. | Hylidae | Ele. | Lep. | Bufonidae |
|------------|--------------|-------|---------|------|--------|---------|------|------|---------|------|------|-----------|
| Pipidae    |              |       |         |      |        |         |      |      |         |      |      |           |
| Meg.       |              |       |         |      |        |         |      |      |         |      |      |           |

**Eye size (mm)**

- 16
- 8
- 4

**Mean eye diameter (mm)**

- 16
- 12
- 8
- 4

**Eye investment (mass)**

- 1.6
- 1.0
- 0.63
- 0.4

**Eye investment (S/L)**

- 1.6
- 1.0
- 0.63
- 0.4

**Corneal investment**

- 1.3
- 1.0
- 0.79

**Larval habitat:**

- Lentic water
- Lotic water
- No larvae
- Obscured
- Terrestrial

**Species:**

- Ingerophrynus galeatus
- Epidalea calamita
- Leptophryne borbonica
- Duttaphrynus stomaticus
- Duttaphrynus melanostictus
- Adenomus kelaartii
- Rentapia hosii
- Phrynoidis asper
- Phrynoidis juxtasper
- Altiphrynoides osgoodi
- Schismaderma carens
- Incilius nebulifer
- Incilius luetkenii
- Anaxyrus punctatus
- Rhinella ocellata
- Rhinella marina
- Atelopus senex
- Leptodactylus melanonotus
- Leptodactylus albilabris
- Lithodytes lineatus
- Physalaemus cuvieri
- Engystomops pustulosus
- Odontophrynus americanus
- Thoropa miliaris
- Teratohyla spinosa
- Espadarana prosoblepon
- Diasporus diastema
- Craugastor fitzingeri
- Barycholos pulcher
- Brachycephalus ephippium
- Allobates femoralis
- Dendrobates auratus
- Dryophytes arenicolor
- Acris gryllus
- Pseudis paradoxa
- Dendropsophus parviceps
- Boana fasciata
- Aplastodiscus albofrenatus
- Nyctimystes papua
- Nyctimystes kubori
- Ranoidea platycephala
- Agalychnis dacnicolor
- Agalychnis callidryas
- Pithecopus hypochondrialis
- Gastrotheca cornuta
- Flectonotus fitzgeraldi
- Cryptobatrachus boulengeri
- Ceratophrys ornata
- Lepidobatrachus laevis
- Telmatobius culeus
- Batrachyla leptopus
- Eupsophus roseus
- Myobatrachus gouldii
- Crinia signifera
- Mixophyes fasciolatus
- Calyptocephalella gayi
- Lithobates catesbeianus
- Rana chensinensis
- Rana temporaria
- Odorrana hosii
- Nidirana chapaensis
- Sylvirana guentheri
- Amolops ricketti
- Pelophylax ridibundus
- Nyctibatrachus humayuni
- Rhacophorus dennysi
- Kurixalus odontotarsus
- Micrixalus phyllophilus
- Euphlyctis cyanophlyctis
- Fejervarya limnocharis
- Occidozyga laevis
- Occidozyga lima
- Platymantis hazelae
- Cornufer guppyi
- Pyxicephalus edulis
- Aubria subsigillata
- Strongylopus grayii
- Ptychadena oxyrhynchus
- Odontobatrachus natator
- Astylosternus montanus
- Nyctibates corrugatus
- Arthroleptis variabilis
- Arthroleptis poecilonotus
- Leptodactylodon ovatus
- Leptopelis parkeri
- Hyperolius bolifambae
- Phlyctimantis verrucosus
- Probreviceps loveridgei
- Spelaeophryne methneri
- Breviceps mossambicus
- Hemisus marmoratus
- Kaloula pulchra
- Kaloula conjuncta
- Uperodon systoma
- Uperodon globulosus
- Chaperina fusca
- Dyscophus antongilii
- Cophixalus cheesmanae
- Mantophryne lateralis
- Hylophorbus rufescens
- Gastrophrynoides borneensis
- Kalophrynus interlineatus
- Anodonthyla boulengerii
- Stumpffia ?grandis
- Plethodontohyla notosticta
- Cophyla tuberifera
- Gastrophryne olivacea
- Ctenophryne aterrima
- Chiasmocleis albopunctata
- Synapturanus mirandaribeiroi
- Hoplophryne rogersi
- Phrynomantis microps
- Nasikabatrachus sahyadrensis
- Sooglossus sechellensis
- Heleophryne purcelli
- Megophrys microstoma
- Megophrys nasuta
- Megophrys feae
- Scutiger boulengeri
- Leptobrachium chapaense
- Pelobates syriacus
- Spea multiplicata
- Scaphiopus holbrookii
- Scaphiopus couchii
- Xenopus fraseri
- Pseudhymenochirus merlini
- Pipa pipa
- Rhinophrynus dorsalis
- Bombina microdeladigitora
- Barbourula busuangensis
- Ascaphus truei
- Leiopelma hochstetteri
Figure S9. Anuran eye size, eye investment relative to mass, eye investment relative to snout-vent length (SVL), and corneal investment relative to eye size across larval habitats. (A) Phylogeny adapted from [36] showing species means for both absolute eye diameter and eye investment relative to body mass across the phylogeny. Bars and phylogeny tips are colored by mating habitat. Meg. = Megophryidae, Brev. = Brevicepitidae, Arthro. = Arthroleptidae, Pyx. = Pyxicephalidae, Dicro. = Dicroglossidae, Myo. = Myobatrachidae, Hem. = Hemiphractidae, Ele. = Eleutherodactylidae, Lep. = Leptodactylidae. (B) Absolute eye size, (C) eye investment relative to mass, (D) eye investment relative to SVL and (E) cornea investment relative to eye size. Black diamonds indicate the means and black bars the medians of each larval habitat state. Kruskal-Wallis tests treating species as independent showed significant differences in absolute eye size and cornea investment relative to eye size, but no significant difference in eye investment relative to mass or eye investment relative to SVL among larval habitats. Phylogenetic ANCOVAs indicated that larval habitat has no significant effects on eye size relative to mass, eye size relative to SVL, or cornea size relative to eye size.
Table S7. Results of non-parametric Kruskal-Wallis tests for differences in absolute eye diameters, eye investments relative to body mass, eye investments relative to snout-vent length, and corneal investments relative to eye diameter across states for six ecological traits. Eye investments are corrected for phylogeny and allometry with body size; cornea investments are corrected for phylogeny and allometry with eye size; species are treated as independent. Significant results are bolded.

| Ecological factor               | Metric               | Adult habitat | Activity period | Mating habitat | Sexual dichromatism | Life history | Larval habitat |
|--------------------------------|----------------------|---------------|-----------------|----------------|---------------------|--------------|----------------|
| Eye diameter                   | \( \chi^2 \)         | 36.3          | 6.37            | 24.6           | 3.20                | 6.94         | 10.5           |
|                                | df                   | 5             | 2               | 3              | 1                   | 1            | 4              |
|                                | p-value              | \(<0.0001\)   | 0.04            | \(<0.0001\)    | 0.07                | \(0.008\)    | 0.03           |
| Eye investment (mass)          | \( \chi^2 \)         | 68.3          | 2.07            | 28.6           | 3.19                | 0.50         | 7.89           |
|                                | df                   | 5             | 2               | 3              | 1                   | 1            | 4              |
|                                | p-value              | \(<0.0001\)   | 0.36            | \(<0.0001\)    | 0.07                | 0.48         | 0.10           |
| Eye investment (SVL)           | \( \chi^2 \)         | 49.9          | 0.95            | 14.6           | 2.17                | 0.28         | 7.5            |
|                                | df                   | 5             | 2               | 3              | 1                   | 1            | 4              |
|                                | p-value              | \(<0.0001\)   | 0.62            | \(0.002\)      | 0.14                | 0.60         | 0.11           |
| Corneal investment             | \( \chi^2 \)         | 8.95          | 1.78            | 15.1           | 0.33                | 4.41         | 10.4           |
|                                | df                   | 5             | 2               | 3              | 1                   | 1            | 4              |
|                                | p-value              | 0.11          | 0.41            | \(0.002\)      | 0.57                | \(0.04\)     | \(0.03\)       |
Table S8. Results of phylogenetic generalised least squares (PGLS) models of transverse eye diameter (ED) vs. each ecological trait with the cube root of mass (RM) as a covariate. Significant effects are bolded. No individual trait states or interactions in coefficients were significant except in the model including mating habitat, which showed a positive interaction between RM and the lotic water mating habitat (p = 0.04) and a positive interaction between RM and the plants mating habitat (0.03).

| Model/factors                  | DF   | Sum Sq. | Mean Sq. | F-value | p-value |
|-------------------------------|------|---------|----------|---------|---------|
| **ED ~ RM * Adult habitat**   |      |         |          |         |         |
| RM                            | 1, 203 | 7.70   | 7.70     | 1037.6  | <0.0001 |
| Adult habitat                 | 5, 203 | 0.46   | 0.09     | 12.38   | <0.0001 |
| RM x Adult habitat            | 5, 203 | 0.09   | 0.02     | 2.30    | 0.046   |
| **ED ~ RM * Activity period** |      |         |          |         |         |
| RM                            | 1, 170 | 6.21   | 6.21     | 754.7   | <0.0001 |
| Activity period               | 2, 170 | 0.82   | 0.41     | 49.6    | <0.0001 |
| RM x Activity period          | 2, 170 | 0.005  | 0.002    | 0.29    | 0.75    |
| **ED ~ RM * Mating habitat**  |      |         |          |         |         |
| RM                            | 1, 170 | 7.33   | 7.33     | 665.1   | <0.0001 |
| Mating habitat                | 3, 170 | 0.12   | 0.04     | 3.71    | 0.01    |
| RM x Mating habitat           | 3, 170 | 0.07   | 0.02     | 2.08    | 0.10    |
| **ED ~ RM * Sexual dichromatism** | |         |          |         |         |
| RM                            | 1, 172 | 8.37   | 8.37     | 679.4   | <0.0001 |
| Sexual dichromatism           | 1, 172 | 0.007  | 0.007    | 0.55    | 0.46    |
| RM x Sexual dichromatism      | 1, 172 | 0.01   | 0.01     | 1.12    | 0.29    |
| **ED ~ RM * Life history**    |      |         |          |         |         |
| RM                            | 1, 189 | 9.27   | 9.27     | 761.1   | <0.0001 |
| Life history                  | 1, 189 | 0.01   | 0.01     | 1.00    | 0.32    |
| RM x Life history             | 1, 189 | 0.001  | 0.001    | 0.11    | 0.74    |
| **ED ~ RM * Larval habitat**  |      |         |          |         |         |
| RM                            | 1, 166 | 8.99   | 8.99     | 692.3   | <0.0001 |
| Larval habitat                | 4, 166 | 0.05   | 0.01     | 1.05    | 0.38    |
| RM x Larval habitat           | 4, 166 | 0.06   | 0.01     | 1.13    | 0.34    |
**Table S9.** Results of phylogenetic generalised least squares (PGLS) models of transverse eye diameter (ED) vs. each ecological trait with the cube root of mass (SVL) as a covariate. Significant effects are bolded.

| Model/factors                        | DF   | Sum Sq. | Mean Sq. | F-value | p-value |
|--------------------------------------|------|---------|----------|---------|---------|
| ED ~ SVL * Adult habitat             |      |         |          |         |         |
| SVL                                  | 1, 208 | 9.93   | 9.93     | 1339.5  | <0.0001 |
| Adult habitat                        | 5, 208 | 0.28   | 0.06     | 7.58    | <0.0001 |
| SVL x Adult habitat                  | 5, 208 | 0.06   | 0.01     | 1.63    | 0.15    |
| ED ~ SVL * Activity period            |      |         |          |         |         |
| SVL                                  | 2, 173 | 0.77   | 0.38     | 61.8    | <0.0001 |
| Activity period                      | 1, 173 | 6.29   | 6.29     | 1013.9  | <0.0001 |
| SVL x Activity period                | 2, 173 | 0.0022 | 0.0011   | 0.18    | 0.84    |
| ED ~ SVL * Mating habitat            |      |         |          |         |         |
| SVL                                  | 1, 172 | 7.82   | 7.82     | 891.34  | <0.0001 |
| Mating habitat                       | 3, 172 | 0.06   | 0.02     | 2.24    | 0.09    |
| SVL x Mating habitat                 | 3, 172 | 0.03   | 0.01     | 1.27    | 0.28    |
| ED ~ SVL * Sexual dichromatism       |      |         |          |         |         |
| SVL                                  | 1, 173 | 8.18   | 8.18     | 865.15  | <0.0001 |
| Sexual dichromatism                  | 1, 173 | 0.001  | 0.001    | 0.15    | 0.70    |
| SVL x Sexual dichromatism            | 1, 173 | 0.01   | 0.01     | 1.51    | 0.22    |
| ED ~ SVL * Life history               |      |         |          |         |         |
| SVL                                  | 1, 191 | 10.4   | 10.4     | 1065.0  | <0.0001 |
| Life history                         | 1, 191 | 0.01   | 0.01     | 1.00    | 0.32    |
| SVL x Life history                   | 1, 191 | 0.01   | 0.01     | 1.34    | 0.25    |
| ED ~ SVL * Larval habitat            |      |         |          |         |         |
| SVL                                  | 1, 168 | 9.37   | 9.37     | 922.2   | <0.0001 |
| Larval habitat                       | 4, 168 | 0.03   | 0.008    | 0.75    | 0.56    |
| SVL x Larval habitat                 | 4, 168 | 0.10   | 0.02     | 2.37    | 0.05    |
**Table S10.** Results of phylogenetic generalised least squares (PGLS) models of transverse cornea diameter (CD) vs. each ecological trait, with transverse eye diameter (ED) as a covariate. Significant effects are bolded.

| Model/factors | DF       | Sum Sq. | Mean Sq. | F-value | p-value |
|---------------|----------|---------|----------|---------|---------|
| CD ~ ED * Adult habitat |          |         |          |         |         |
| ED            | 1, 206   | 3.7     | 3.7      | 7694.9  | <0.0001 |
| Adult habitat | 5, 206   | 0.01    | 0.003    | 5.14    | 0.0002  |
| ED x Adult habitat | 5, 206 | 0.006   | 0.001    | 2.63    | 0.03    |
| CD ~ ED * Activity period |       |         |          |         |         |
| ED            | 1, 172   | 2.67    | 2.67     | 4921.2  | <0.0001 |
| Activity period | 2, 172 | 0.10    | 0.05     | 96.67   | <0.0001 |
| ED x Activity period | 2, 172 | <0.001  | 0.0001   | 0.34    | 0.71    |
| CD ~ ED * Mating habitat |          |         |          |         |         |
| ED            | 1, 170   | 2.75    | 2.75     | 4893.6  | <0.0001 |
| Mating habitat | 3, 170  | 0.009   | 0.003    | 5.15    | 0.002   |
| ED x Mating habitat | 3, 170 | 0.0007  | 0.0002   | 0.42    | 0.74    |
| CD ~ ED * Sexual dichromatism |       |         |          |         |         |
| ED            | 1, 171   | 2.93    | 2.93     | 4880.9  | <0.0001 |
| Sexual dichromatism | 1, 171 | 0.00001 | 0.00001  | 0.01    | 0.91    |
| ED x Sexual dichromatism | 1, 171 | 0.00005 | 0.00005  | 0.08    | 0.78    |
| CD ~ ED * Life history |          |         |          |         |         |
| ED            | 1, 189   | 3.29    | 3.29     | 4653.1  | <0.0001 |
| Life history  | 1, 189   | 0.001   | 0.001    | 1.65    | 0.20    |
| ED x Life history | 1, 189 | 0.0006  | 0.0006   | 0.80    | 0.37    |
| CD ~ ED * Larval habitat |          |         |          |         |         |
| ED            | 1, 166   | 3.01    | 3.01     | 3985.8  | <0.0001 |
| Larval habitat | 4, 166  | 0.002   | 0.0005   | 0.60    | 0.66    |
| ED x Larval habitat | 4, 166 | 0.005   | 0.001    | 1.65    | 0.16    |
**Figure S10.** Comparison of our data with those from a paper measuring cornea-body size allometry in fresh frogs [28]. We compared our full museum specimen dataset, a subset of our museum data matching the families sampled in the other study, our fresh specimen dataset, and the fresh specimen dataset from the other study with standardized major axis (SMA) tests for differences in slopes and intercepts. **(A)** Corneal scaling with the cube root of mass shows no difference in slopes among datasets (Likelihood ratio stat.: 2.52, df = 3, p = 0.47) but does show a significant difference in intercepts (Wald stat.: 46.8, df = 3, p < 0.0001). **(B)** Corneal scaling with snout-vent length (SVL) shows no difference in slopes among datasets (Likelihood ratio stat.: 1.90, df = 3, p = 0.59) but a significant difference in intercepts (Wald stat.: 102.6, df = 3, p < 0.0001).
Figure S11. Recategorization of our species data into the binary habitat states used by another study of frog corneal size [28] replicates their finding that relative eye (corneal) size/investment does not differ across habitats. (A) Absolute eye diameter is significantly different across binary habitat states ($\chi^2 = 8.18$, df = 1, $p = 0.004$). (B) Eye investment relative to mass ($\chi^2 = 0.052$, df = 1, $p = 0.82$) and (C) eye investment relative to SVL ($\chi^2 = 8.18$, df = 1, $p = 0.36$) do not significantly differ across binary habitat states. Aq/S = aquatic and semiaquatic species and T/Ar = terrestrial and arboreal species according to states defined by Huang et al [28]. Black bars indicate the medians and black diamonds the means for each state. Points are colored by the finer-resolution adult habitat categorizations we used in this study.
Electronic supplementary material: Eye size and investment in frogs and toads (Thomas et al.)

Supplemental References

Full references used in assigning each anuran species to ecological traits/states (Table S1)

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Personal observations used in assigning each anuran species to ecological traits/states (Table S1)

AC = Alan Channing (University of the Western Cape)
BLS = Bryan L. Stuart (North Carolina Museum of Natural Sciences)
DJG = David Gower (Natural History Museum)
DV = Deepak Veerappan (Natural History Museum)
FK = Fred Kraus (University of Michigan)
JL = Jim Labisko (University of Kent)
JWS = Jeff Streicher (Natural History Museum)
MOR = Mark-Oliver Rödel (Museum für Naturkunde, Berlin)
RCB = Rayna C. Bell (Smithsonian National Museum of Natural History)
RDS = Rafael de Sá (University of Richmond)
SM = Stephen Mahoney (Natural History Museum)
SPL = Simon Loader (Natural History Museum)
YC = Yodchaiy Chuaynkern (Khon Kaen University)