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Evolution of the third eye: a phylogenetic comparative study of parietal-eye size as an ecophysiological adaptation in *Liolaemus* lizards

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The parietal, or third, eye is a photosensory organ situated in the middle of the skull of many lizards. Despite many hypotheses, its exact ecological functions are still unclear. Studies have compared the presence and absence of a functioning parietal eye, although there are no quantitative studies of parietal-eye traits in relation to ecology, physiology or behaviour. In the present study, we report the first comparative study of relative parietal-eye size in relation to climatic and thermophysiological variables. We studied thirty species of *Liolaemus*, a genus of South-American lizards inhabiting a range of climatic conditions, but found little evidence for adaptation to thermal environment, in that parietal-eye size did not vary meaningfully with latitude, altitude or any measures of environmental temperature. Neither did it relate to thermophysiology; there was a weak relation to thermal tolerance, although this was partially confounded with body size, which explained 23% of the among-species variance after controlling for within-species variation. The negative results obtained could not be explained by phylogenetic constraints because we found no evidence of phylogenetic inertia. We also observed high intraspecific variation indicating that parietal-eye size may not be under strong selection for accuracy. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **101**, 870–883.

ADDITIONAL KEYWORDS: adaptive accuracy – comparative method – orientation – photoreception – thermophysiology – thermoregulation.

INTRODUCTION

The parietal, or ‘third’, eye is a photosensory organ that occurs as a part of the pineal complex (i.e. the pineal gland and associated structures) in some vertebrates, most notably in many lizards and in the tuatara (Eakin, 1973). Almost all vertebrates, except crocodilians and a few mammals, have a pineal complex that is generally involved in the endocrinal regulation of circadian and seasonal cycles, reproduction, and body temperature (Quay, 1979; Ralph *et al.*, 1979); its main secretory product being the hormone melatonin (Lutterschmidt, Lutterschmidt & Hutchison, 2003). The pineal complex is considered to be most developed in ‘lower’ vertebrates and to show a tendency for evolutionary reduction in size and functionality (Edinger, 1955; Eakin, 1973; but see also Quay, 1979). The ancestral state is presumed to have been a possibly paired photosensory organ, as seen in some extant cyclostomes. Possibly, the parietal eye and the pineal gland of tetrapods are the descendants of the left and right parts of this organ (Eakin, 1973). In the pineal gland, the endosecretory pinealocytes appear to be derived from photoreceptor cells (Collin, 1971; Kappers, 1971; Ralph *et al.*, 1979) and, in many taxa, including lizards, the pineal gland retains photosensory capability (Edinger, 1955; Gundy & Wurst, 1976b; Quay, 1979).

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The parietal eye shows a phylogenetic distribution that reflects frequent reduction, loss or rudimentation. Indeed, several Permian reptiles, including some therapsids, had parietal foramina that are relatively much larger than those of any extant taxa (Edinger, 1955; Quay, 1979). The parietal eye is lost in birds, turtles, crocodilians, snakes, and mammals, as well as in many individual species, genera, and families of lizards. Gundy & Wurst (1976a, b) report that about 60% of all lizard genera include species with an externally visible parietal eye. Among amphibians, a frontal eye occurs in ranid frogs, although parietal eyes appear absent or vestigial in other taxa (Ralph, 1975). The loss of parietal eyes is also supported by reports of possible developmental vestiges in some snakes, birds, and mammals (Stebbins & Eakin, 1958; Quay, 1979).

The lizard parietal eye (Fig. 1) clearly has photosensory capability as indicated by its structure with a somewhat cup-shaped photosensory retina usually below a translucent 'lens' and a 'cornea', and by direct evidence for electrophysiological response to light (Hamasaki, 1969; Solessio & Engbretson, 1999). Nevertheless, its specific ecological functions are enigmatic (Eakin, 1973). The frequent evolutionary losses could mean that its functionality is easily dispensable, and one hypothesis is that the parietal eye is a functionless vestige that is maintained with some degree of complexity in some taxa as a result of unknown constraints. As a result of its complex cup-eye design, however, the parietal eye must have had an adaptive ancestral photosensory function, and alternative adaptive explanations of its maintenance can be found either in continuation of ancestral function(s), or in the exaptation of new functions. Evidence for current adaptation in the broad sense can either be found in direct demonstration of ecologically relevant functionality, or indirectly through meaningful covariation of parietal-eye traits with ecological variables on low phylogenetic levels.

Figure 1. Two images of parietal eyes in *Liolaemus nitidus*. The arrows point to the parietal eyes situated in the parietal scale. In the right-hand image, the outline of the eye is indicated with a line. Note the difference in parietal-eye size in these two individuals. Left image: courtesy of Óscar Acevedo; right image: courtesy of Javiera Constanzo.

Direct evidence for functionality has been sought in a number of studies where the parietal or frontal eyes of frogs, lizards, or the tuatara have been removed or occluded (Tosini, 1997), although the results are often complex, mixed or ambiguous. Still, the apparent functionality and structural characteristics of the parietal photoreceptors in lizards provide the basis for several adaptive hypotheses. First, a potential function of a photoreceptive organ is to measure light intensity, and several studies have considered the hypothesis that the parietal eye functions as an illuminometer or as a radiation dosimeter (Glaser, 1958; Stebbins & Eakin, 1958; Packard & Packard, 1972; Eakin, 1973; Hutchison & Kosh, 1974; for a critique of this hypothesis, see Dodt, 1973; Ralph *et al.*, 1979). A radiation dosimeter could function in seasonal behaviour and physiology (e.g. in the timing of reproduction) but could also have a role in thermoregulation. A thermoregulatory role for the parietal eye is supported by evidence that parietalectomized lizards show alterations in their preferred body temperatures and basking behaviours, although the exact effects may interact with season and time of day (Ralph *et al.*, 1979; Tosini, 1997), and the exact thermoregulatory function, if any, of the eye is still unclear. Second, the parietal eyes of several lizards are sensitive to different wavelengths (Jenison & Nolte, 1980), and Solessio & Engbretson (1993) found responses to blue and green/red light to be situated within the same photoreceptor cells and to act antagonistically by eliciting a neural response during the light conditions of dawn and dusk, and they proposed the hypothesis that the parietal eye acts as a dawn–dusk detector. Finally, there is evidence that the parietal eye is sensitive to polarized light (Beltrami *et al.*, 2010), and occlusion of the parietal eye has been shown to specifically disrupt sun-compass orientation in *Podarcis sicula* (Foa *et al.*, 2009; Beltrami *et al.*, 2010), as well as general orientation or homing ability of displaced individuals from several other species (Adler & Phillips, 1985; Ellis-Quinn & Simon, 1991; Freake, 1999, 2001). This suggests that the lizard parietal eye may function in orientation. Because there is no evidence that the lateral eyes of lizards are sensitive to polarized light, the hypothesis that the orientation is based on polarized light is also attractive in that it provides an explanation for the maintenance of the parietal eye through a functional specialization different from those of the lateral eyes.

Edinger (1955) asked 'is the size of the parietal organ correlated with the habitat of the reptile?', but there have been few attempts at answering her question. The only information we know comes from studies of Gundy, Ralph & Wurst (1975) and Ralph (1975), who found that lizard genera lacking the parietal eye tend to have more equatorial geographic distributions. On the basis of this, and on the observation of very large pineal glands in some high-latitude mammals, it was hypothesized that the presence of a complex pineal organ in tetrapods in general, and the parietal eye of lizards specifically, allows animals to adapt more easily to more variable seasonal and/or diurnal cues at higher latitudes (Gundy *et al*., 1975; Ralph, 1975; Gundy & Wurst, 1976a; Ralph *et al*., 1979). Alternatively, the pattern could result from a thermoregulatory role of the parietal eye if thermoregulation is more challenging at higher latitudes.

Tabulations of presence or absence of an organ across higher taxonomic levels is, however, a very approximate test of ecological relevance, and there has been no control for the effects of phylogeny. If parietal eyes really do have adaptive functions related to either thermoregulation or to seasonal or diurnal behaviour, then we could expect their size to covary with thermal environment, thermophysiology, habitat, climate or seasonality.

In the present study, we provide the first quantitative data relating to Edinger's (1955) question, and the first comparative tests of at least some of the many adaptation hypotheses for the parietal eye. We measured the size of the parietal-eye aperture in thirty species of *Liolaemus*, a South American genus of Iguanid lizards with a wide distribution both latitudinally and altitudinally. After describing the pattern of within-species variation, we test whether parietal-eye size is related to latitude across species, as in the previously proposed hypothesis (Gundy *et al*., 1975; Ralph, 1975; Gundy & Wurst, 1976a; Ralph *et al*., 1979). We refine this to look for climatic and altitudinal effects (as suggested by Quay, 1979), as well as for effects of environmental temperature. We further test hypotheses about an adaptive role in thermoregulation by studying the relation of parietal-eye size to thermophysiological variables. Finally, we discuss data for a few species on parietal-eye size in relation to home-range size to provide some preliminary tests of the hypothesis that the parietal eye is involved in spatial orientation.

MATERIAL AND METHODS

Data for parietal-eye sizes as well as parietal-scale size, head length and width, and snout–vent length were obtained for one or more individuals from thirty species of *Liolaemus* (Table 1). We discarded the species *Liolaemus vallecurensis* from the comparative analyses, however, because we only had data from a single juvenile individual. Lengths and area of the parietal eye and the parietal scale were measured from photographs of the lizard's head (Fig. 2) using IMAGE PRO PLUS, version 4.5 (Media Cybernetics). Photographs were taken using a Nikon Microscopy Unit (Nikon Corp.) coupled to a digital recording system (CoolSNAP-Procf; Media Cybernetics). Measures from one *L. ornatus* and two *L. bisignatus* were not included as a result of the formation of plaque that obscured the outline of the eye. Body measurements were taken with a caliper (±1 mm). We measured head width at the widest part of the head at the level of the ears, head length from the ear to the tip of the nose, and snout–vent length from the tip of the nose to the border of the cloaca. We removed the allometric effects of body size by regressing (natural) log parietal-eye area on log head width in an analysis of covariance (ANCOVA) including species as a factor. Because of low sample sizes for individual species, it was necessary to use a common within-species allometric coefficient for all species. We used the estimated species intercepts from this ANCOVA as data for the comparative study. We repeated all analyses using head length and snout–vent length instead of head width as a size measure, and obtained essentially identical results.

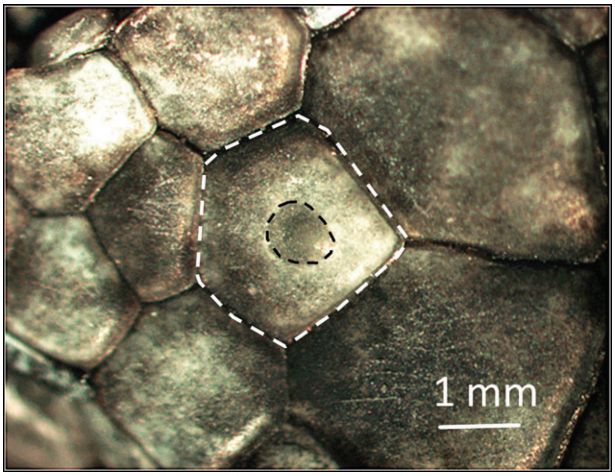

Figure 2. Picture of parietal scale with parietal eye in *Liolaemus bisignatus*: The outline of the scale and the eye are indicated by lines. Measurements of their areas were obtained by drawing an outline with IMAGE PRO PLUS, version 4.5, which then automatically computed the enclosed area. Image courtesy of Beatriz López.

For the comparative analyses, we used several different predictor variables (Table 2). These include the species-range midpoints of latitude and altitude obtained from Núñez (1992) and Espinoza, Wiens & Tracy (2004), as well as a thermal index, TI, calculated from the latitude and altitude *sensu* Espinoza

Table 1. Species means and standard deviations for morphological traits used in the present study

		Parietal eye area (mm^2)	Parietal scale area (mm^2)	Head width (mm)	Head length (mm)	Snout–vent length (mm)	Size-corrected lnPE	Size-corrected lnPS
Liolaemus	*N*	(mean, SD)	(mean, SD)	(mean, SD)	(mean, SD)	(mean, SD)	(±SE)	(±SE)
alticolor	7	0.062 ± 0.016	1.06 ± 0.24	6.99 ± 1.18	10.31 ± 0.67	47.11 ± 3.73	–3.96 ± 0.52	–0.98 ± 0.56
bellii	6	0.079 ± 0.034	0.93 ± 0.27	10.45 ± 0.80	14.82 ± 1.19	70.02 ± 4.56	–3.99 ± 0.63	–1.34 ± 0.44
bibronii	1	0.089 ± –	1.34 ± –	7.8 ± –	10.5 ± –	51.5 ± –	–3.64 ± 0.65	–0.79 ± 0.45
bisignatus	15	0.114 ± 0.042	1.10 ± 0.33	9.45 ± 1.28	14.16 ± 2.20	57.25 ± 9.52	–3.55 ± 0.59	–1.14 ± 0.41
constanzae	6	0.102 ± 0.023	1.28 ± 0.27	10.48 ± 1.87	13.45 ± 1.36	61.53 ± 4.54	–3.69 ± 0.63	–1.00 ± 0.44
curis	2	0.175 ± 0.167	1.25 ± 0.53	13.35 ± 3.18	17.20 ± 3.25	76.05 ± 17.47	–3.57 ± 0.72	–1.18 ± 0.50
cyanogaster	2	0.160 ± 0.023	1.04 ± 0.23	7.65 ± 1.06	11.25 ± 2.33	47.40 ± 12.87	–3.04 ± 0.59	–1.05 ± 0.41
dorbignyi	1	0.086 ± –	1.00 ± –	16.0 ± –	19.6 ± –	86.3	–4.10 ± 0.81	–1.46 ± 0.56
eleodori	10	0.157 ± 0.049	1.38 ± 0.28	10.21 ± 1.08	14.01 ± 1.54	60.99 ± 7.18	–3.27 ± 0.62	–0.92 ± 0.43
fabiani	16	0.191 ± 0.045	1.59 ± 0.25	10.83 ± 0.87	15.91 ± 1.44	71.22 ± 5.87	–3.09 ± 0.63	–0.80 ± 0.44
fitzgeraldi	10	0.112 ± 0.035	1.04 ± 0.27	7.82 ± 0.52	11.30 ± 0.95	51.28 ± 3.35	–3.45 ± 0.55	–1.08 ± 0.38
hellmichi	4	0.039 ± 0.022	0.69 ± 0.13	7.50 ± 0.65	10.68 ± 0.64	45.40 ± 2.71	–4.53 ± 0.55	–1.48 ± 0.39
hernani	6	0.076 ± 0.054	0.75 ± 0.11	7.55 ± 0.95	11.87 ± 1.20	51.42 ± 4.23	–3.96 ± 0.55	–1.35 ± 0.38
jamesi	7	0.284 ± 0.102	1.63 ± 0.39	15.16 ± 1.39	19.26 ± 1.27	88.50 ± 4.00	–2.92 ± 0.72	–0.97 ± 0.50
lemniscatus	6	0.042 ± 0.007	0.63 ± 0.038	5.72 ± 0.59	9.58 ± 1.01	43.27 ± 5.17	–4.20 ± 0.48	–1.37 ± 0.33
lorenzmuelleri	6	0.104 ± 0.036	1.51 ± 0.33	12.78 ± 1.72	17.22 ± 1.29	77.73 ± 3.98	–3.83 ± 0.68	–0.95 ± 0.47
maldonadae	5	0.191 ± 0.041	2.01 ± 0.37	12.80 ± 1.25	18.22 ± 1.08	77.42 ± 6.76	–3.18 ± 0.68	–0.66 ± 0.47
monticola	2	0.081 ± 0.050	0.83 ± 0.13	8.40 ± 0.28	12.80 ± 1.27	57.25 ± 2.05	–3.88 ± 0.61	–1.31 ± 0.42
nigromaculatus	2	0.085 ± 0.020	1.69 ± 0.10	10.50 ± 1.41	14.45 ± 0.21	58.40 ± 0.71	–3.87 ± 0.66	–0.71 ± 0.46
nigroroseus	2	0.103 ± 0.061	0.98 ± 0.015	8.65 ± 0.78	13.00 ± 0.00	58.15 ± 2.19	–3.64 ± 0.62	–1.15 ± 0.43
nigroviridis	5	0.136 ± 0.073	2.16 ± 1.04	11.14 ± 0.47	15.56 ± 0.79	72.14 ± 5.05	–3.51 ± 0.65	–0.59 ± 0.45
nitidus	10	0.204 ± 0.057	2.43 ± 0.53	12.41 ± 1.96	18.02 ± 1.96	81.60 ± 7.71	–3.11 ± 0.66	–0.46 ± 0.46
ornatus	8	0.092 ± 0.037	1.15 ± 0.30	10.06 ± 0.92	12.80 ± 1.42	60.73 ± 4.76	–3.83 ± 0.62	–1.11 ± 0.43
pictus	2	0.113 ± 0.033	1.29 ± 0.42	10.30 ± 4.53	12.50 ± 1.98	50.60 ± 5.09	–3.56 ± 0.65	–0.97 ± 0.45
platei	9*	0.073 ± 0.018	1.00 ± 0.16	7.50 ± 0.75	11.26 ± 0.94	49.56 ± 4.26	–3.80 ± 0.54	–1.07 ± 0.38
pseudolemniscatus	1	0.068	0.79	6.2	10.3	49.5	–3.77 ± 0.60	–1.20 ± 0.41
schroederi	2	0.113 ± 0.005	1.28 ± 0.048	7.30 ± 0.57	12.40 ± 0.71	55.85 ± 0.35	–3.36 ± 0.58	–0.80 ± 0.40
tenuis	5	0.079 ± 0.035	0.93 ± 0.29	7.52 ± 0.48	11.76 ± 1.27	49.10 ± 5.59	–3.83 ± 0.55	–1.19 ± 0.38
vallecurensis	1†	0.039	0.60	5.7	8.2	35.0	–4.28 ± 0.58	–1.42 ± 0.40
walkeri	2	0.064 ± 0.011	0.70 ± 0.027	6.10 ± 0.57	8.65 ± 0.71	36.40 ± 0.85	–3.83 ± 0.54	–1.30 ± 0.37
Mean		0.110	1.20	9.47	13.37	59.29	–3.67	–1.06
CV		50.4%	37.5%	29.9%	23.3%	24.0%	38.3%‡	26.5%‡

The size-corrected lnPE and lnPS columns give the estimated mean species values of the natural log of the parietal-eye and parietal-scale areas, respectively, from a model that includes species and the natural log of head width. The estimated allometric exponents from these models are 0.592 ± 0.261 and 0.526 ± 0.181, respectively.

*Only eight individuals of *L. platei* for head length, head width and snout–vent length.

†Juvenile individual, and *L. vallecurensis* was not used in the comparative analyses.

‡Computed as the standard deviation because these are on log scale.

Table 2. Geographic and thermal data by species

Liolaemus	Latitude (°S)/ altitude (m) sample location	Latitude (°S) (range)	Altitude (m) (tange)	$T_{\mathrm{sel}}/T_e/T_s/Ct_{\min}$ (°C)
alticolor	18°10′/4350	18.23–19.25	4000–4570	32.92/28.41/28.69/13.12
bellii	33°22′/2353	32.33–36.07	2000–3200	35.10/40.92/28.29/4.70
bibronii	43°32′/168	32.00–49.00	0–3000	35.14/28.33/33.58/13.41
bisignatus	26°09′/710	24.00–27.07	19 m–1500	34.75/33.51/38.13/12.52
constanzae	23°46′/2250	22.92–23.68	2250–3800	34.31/35.30/32.48/11.33
curis	34°57′/1768	34.92–34.97	1520–3000	35.90/32.93/31.50/11.70
cyanogaster	40°57′/700	36.13–39.27	0–1200	33.79/31.40/34.80/12.25
dorbignyi	22°36′/4250	22.03–28.00	2475–3550	31.01/24.68/23.00/12.75
eleodori	27°04′/3670	27.45–27.45	3670–4125	35.87/32.11/36.57/6.42
fabiani	23°23′/2450	22.92–26.77	2000–3000	31.46/29.53/25.93/8.71
fitzgeraldi	32°50′/2901	30.95–32.82	2100–3500	35.68/32.86/35.49/10.04
hellmichi	23°32′/100	24.00–24.00	100–1785	33.69/33.40/28.71/14.60
hermani	34°57′/1768	34.42–34.97	1760–2000	35.25/35.96/32.33/11.64
jamesi	18°10′/4350	17.50–24.00	3000–4600	32.94/26.78/31.30/8.08
lemniscatus	33°35′/890	31.58–39.00	0–2100	35.20/38.06/32.26/12.44
lorenzmuelleri	29°51′/3206	29.85–30.22	2300–3275	36.13/38.92/30.51/10.13
maldonadae	30°43′/2700	30.72–30.72	2600–2800	NA
monticola	33°35′/890	32.03–33.82	500–2200	36.80/36.15/36.18/9.68
nigromaculatus	29°34′/336	26.35–30.00	19–756	35.12/33.04/34.40/8.40
nigroroseus	23°20′/2300	22.33–23.68	2500–4000	34.75/33.36/31.07/8.40
nigroviridis	33°22′/2353	30.48–34.08	500–4000	36.31/40.73/32.25/11.64
nitidus	33°35′/890	28.00–37.70	0–3153	35.71/36.31/34.40/8.88
ornatus	19°15′/3710	18.25–27.62	2000–4800	35.32/37.73/36.93/6.91
pictus	40°57′/700	35.93–42.00	0–1000	34.30/29.64/31.24/8.47
platei	27°03′/200	25.05–31.92	0–1360	34.50/32.77/35.68/11.98
pseudolemniscatus	29°34′/336	29.00–32.00	100–1300	34.60/33.30/31.25/11.08
schroederi	33°22/2353	32.95–39.27	200–2600	34.90/35.97/24.05/NA
tenuis	33°35′/890	32.07–41.92	0–1800	37.20/40.49/30.68/11.65
vallecurensis	29°51′/3206	29.00–29.00	3206–3400	33.76/37.08/34.85/8.20
walkeri	22°36′/4250	22.83–22.92	2450–4250	33.76/24.30/31.00/12.90

The latitude and altitude where the study species were sampled are given first in degrees and minutes for latitude and metres above sea level for altitude. The latitudinal range is given in degrees converted to a decimal scale. Latitudinal and altitudinal ranges for the species are from Núñez (1992), and are not always consistent with our sample. In the final column, the species mean is given for selected body temperature (T_{sel}), operative temperature (T_e), substrate temperature (T_s), and critical thermal minimum ($Ct_{\min}$); more detail, including sample sizes and standard errors of these can be found in Labra (1998) and Labra *et al.* (2008; 2009), with the exception of *L. maldonadae*. NA, not applicable.

et al. (2004). For microclimatic variables, we used the substrate temperature and the operative temperature measured with copper models at the locations where lizards were collected or observed, as described by Labra, Pienaar & Hansen (2009). Thermophysiological variables measured in the laboratory were the selected body temperature, T_{sel}, the temperature lizards chose in a thermal gradient, and the critical thermal minimum, $Ct_{\min}$, recorded by cooling lizards until they were unable to right themselves when turned on their backs (for experimental details see Labra, 1998; Labra, Soto-Gamboa & Bozinovic, 2001; Labra & Bozinovic, 2002; Vidal, Ortiz & Labra, 2008). Data for these predictor variables were first reported by Labra (1998) and Labra *et al.* (2008, 2009) for all species except *L. maldonadae*. Thermophysiological data are not available for *L. maldonadae*.

Comparative analyses require a phylogeny with branch lengths. We built a phylogeny for 22 of our measured species, as shown in Figure 3, based on sequence data from the two mitochondrial genes NADH dehydrogenase subunit 1 (ND1) (1182 bp) and cytochrome *b* (Cytb) (659 bp). These were available

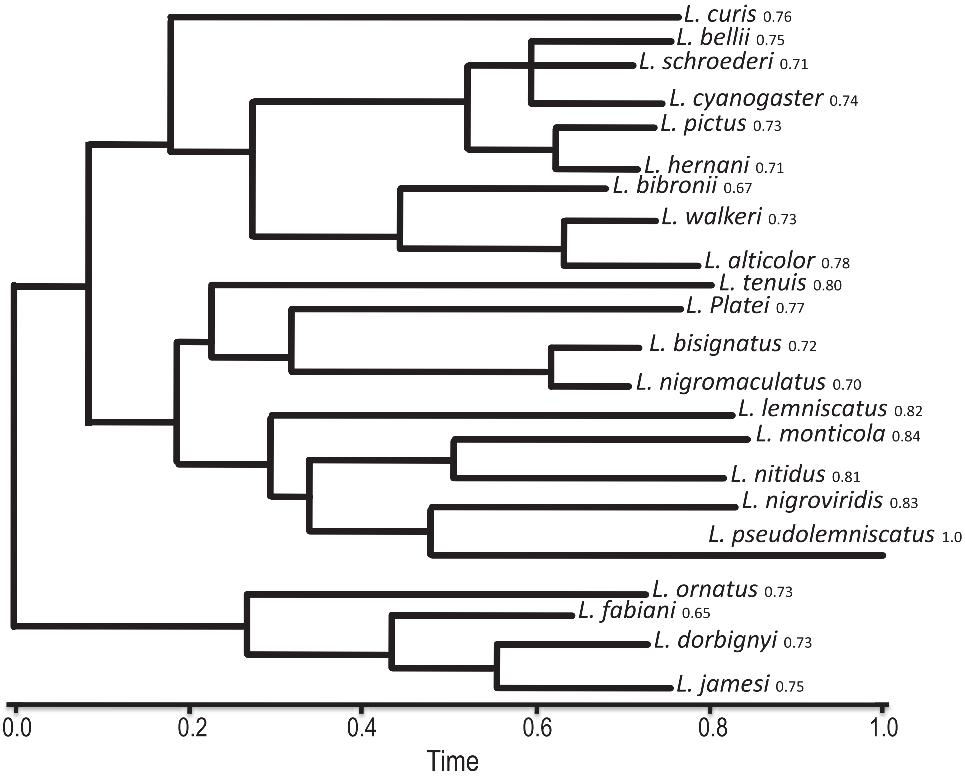

Figure 3. The phylogeny with branch lengths used in this study. Time is measured in units of total tree height (i.e. maximum distance from root to a species). Distance from root is indicated at each node. The phylogeny is built from sequences of two mitochondrial genes, NADH dehydrogenase subunit 1 (ND1) and cytochrome *b* (Cytb), as explained in the main text. (Accession numbers or source for the used sequences*: L. alticolor:* ND1 = AF099218.1; Cytb = –; *L. bellii*: ND1 = AF099223.1, Cytb = –; *L. bibroni*: ND1 = AF099221.1; Cytb = AY173791.1; *L. bisignatus*: ND1 = J. Schulte (pers. comm.), Cytb = –; *L. curis*: ND1 = J. Schulte (pers. comm.), Cytb = –; *L. cyanogaster*: ND1 = –, Cytb = DQ989786.1; *L. dorbignyi*: ND1 = AF099248.1, Cytb = –; *L. fabiani*: ND1 = AF305793.1, Cytb = –; *L. hernani*: ND1 = AY297529.1, Cytb = –; *L. jamesi*: ND1 = AF305788.1; Cytb = –; *L. lemniscatus*: ND1 = AF099229.1, Cytb = EU649137.1; *L. monticola*: ND1 = AF099230.1, Cytb = DQ989787.1; *L. nigromaculatus*: ND1 = AY297526.1, Cytb = EU220834.1; *L. nigroviridis*: ND1 = AF099233.1, Cytb = AY850633.1; *L. nitidus*: ND1 = AF099231.1, Cytb = EU220835.1; *L. ornatus*: ND1 = AF099266.1, Cytb = –; *L. pictus*: ND1 = U82684.1, Cytb = AY367791.1; *L. platei*: ND1 = AY297528.1, Cytb = AY850635.1; *L. pseudolemniscatus*: ND1 = –, Cytb = EU220833.1; *L. schroederi*: ND1 = AF305791.1, Cytb = –; *L. tenuis*: ND1 = AF099228.1, Cytb = DQ989790.1; *L. vallecurensis*: ND1 = –, Cytb = AY367808.1; *L. walkieri*: ND1 = AF305790.1, Cytb = –; *Phymaturus indistinctus*: ND1 = AY661893.1, Cytb = AY367794.2).

for 20 and 11 of our species, respectively (for accession numbers in GenBank, see Fig. 3). Two of the NADH sequences (*L. bisignatus* and *L. curis*) were kindly provided by J. Schulte (Schulte *et al*., 2000; Schulte *et al*., 2004). *Phymaturus indistinctus* was used as outgroup and the final alignment included 23 species, where ten species had data for both genes. Sequence data for *L. alticolor* and *L. jamesi* were based on the closely associated *L. chaltin* and *L. aymararum*, respectively. Sufficient genetic data were not available for *L. constanzae*, *L. eleodori*, *L. fitzgeraldi*, *L. hellmichi*, *L. lorenzmulleri*, *L. maldonadae*, and *L. nigroroseus* and these species were only included in nonphylogenetic analyses.

The phylogeny was constructed using a Bayesian analysis performed in MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001). A general time-reversible model with a gamma distribution (GTR + Γ) fitted the sequences best according to both the Akaike information criterion (AIC) and the small-sample corrected AIC_c in JMODELTEST, version 0.1.1 (Posada, 2008). We used flat priors for all model parameters except the shape parameter of the gamma distribution, for which we used a uniform prior on the range [0, 200]. Two independent analyses were run simultaneously, each starting from different random trees. Each search was run with four Markov chains for three million generations and trees were sampled every 1000th generation. The first 750 generations were discarded as a burn-in. We ensured that stationarity was reached by the end of the burn-in by analyzing time plots of log probability of the data.

Phylogenetic comparative analyses based on the phylogeny in Figure 3 were conducted with SLOUCH software (Hansen *et al.*, 2008; Labra *et al.*, 2009), which is built on a model of adaptive evolution (Hansen, 1997). In essence, this model, which is based on an Ornstein–Uhlenbeck process, assumes that a trait, parietal-eye size in our case, tracks an optimum influenced by the states of one or more predictor variables. The predictor variables can be fixed effects mapped onto the phylogeny (Hansen, 1997; Butler & King, 2004), although here we used a 'random-effects' approach, where mapping of ancestral states was replaced with the assumption that the predictor variables had evolved along the phylogeny according to a Brownian-motion process (Hansen *et al.*, 2008). The SLOUCH software returns estimates of the linear influence of the predictor variables on the optimum, or more precisely on the 'primary' optimum, defined as an optimal state where all ancestral constraints are lost (Hansen, 1997). The model also estimates two parameters describing the adaptive process. The phylogenetic half-life, $t_{1/2}$, is the time it takes for a species evolving in a new niche to have lost on average half the influence of its ancestral state. This is a measure of phylogenetic inertia *sensu* Hansen & Orzack (2005); if $t_{1/2}$ is large relative to branch lengths on the phylogeny we expect species to be further from their current primary optima and to show a stronger residual correlation with each other. We report $t_{1/2}$ in units of total tree height, so that a $t_{1/2} = 1$ means that an extant species is expected to have lost half the ancestral influence from the root of the phylogeny. If the model is fitted without predictor variables, $t_{1/2}$ measures the overall phylogenetic effect or signal in the response variable. The stationary variance, v_y, measures the influence of 'secondary' stochastic factors (e.g. unmeasured selective factors) when they have reached a balance with adaptation to the 'primary' factors that we explicitly included in the model.

SLOUCH allows the inclusion of known measurement variances in both response and predictor variables. Because the intercepts and means are based on observations of one to 16 individuals per species, they are subject to large sampling errors that need be taken into account as measurement variance in the comparative analysis. Because of the low sample sizes per species, we computed weighted average sampling variances across species and divided this by the respective species sample sizes to obtain an estimate of the estimation variance of each species mean (Labra *et al.*, 2009). The measurement variances of the intercepts were based on the residual variances from the ANCOVA divided by species-specific sample size, and are thus conditional on the estimated allometric slope.

We report estimates (±SE) and all statistical analyses were conducted in R, versions 2.8.1 and 2.10.0. We evaluated model fit based on $\mathrm{AIC_c}$, a small-sample version of AIC (Burnham & Anderson, 1998). A justification of model selection by AIC in comparative studies is provided in Butler & King (2004) or Lajeunesse (2009). In the phylogenetic analyses, we counted only $t_{1/2}$, v_y, and the intercept and slopes of the regression in the number of parameters (i.e. we did not count parameters describing the evolution of the predictor variables). In the nonphylogenetic analysis, we counted the intercept and slopes and the residual variance parameter. In the within-species analysis, we approximated standard errors of the standard deviation of residuals as the standard deviation divided by $\sqrt{2N}$, where N is sample size (161) minus the parameters in the model (31).

RESULTS

WITHIN-SPECIES VARIATION

The species means and standard deviations of the parietal-eye area and other morphological characters are reported in Table 1. Because sample sizes for most species are very small, we did not try to interpret species differences in standard deviations. The regression of log parietal-eye area on log head width in a model that includes species-specific intercepts gave an assumed common allometric exponent of 0.59 ± 0.26. Similar regressions on head length and snout–vent length gave allometric exponents of 0.61 ± 0.32 and 0.41 ± 0.32, respectively. The allometric exponent in relation to the area of the parietal scale in which the eye is embedded was 0.63 ± 0.11, which shows that parietal-eye size is not isomorphic to parietal-scale size. The parietal-scale area itself related to head width, head length, and snout–vent length with allometric exponents of 0.53 ± 0.18, 0.76 ± 0.22, and 0.67 ± 0.22, which are approximately similar to those of the parietal eye. These allometries are rather shallow. Note that the allometric exponent of a circular area is expected to be twice the exponent of a corresponding linear dimension. Thus, if parietal-eye area was isometric to the size of the animal we would have expected an allometric exponent of two relative to linear measures of overall size. Also, for comparison, the allometric exponents of head width and head length on snout–vent length, which we expect to be half those of an area, were 0.61 ± 0.09 and 0.81 ± 0.05, respectively.

The common within-species standard deviation of residuals from the allometric relation of log parietal-eye area to the logs of head width, head length, and snout–vent length were 0.325 ± 0.020, 0.327 ± 0.020, and 0.329 ± 0.020, respectively. These numbers are approximately equal to coefficients of variation, CV, of the trait on the original scale [i.e. the standard deviation of log(x) is approximately equal to the coefficient of variation of x]. Comparing these numbers to CVs for quantitative traits as reported in Hansen, Carter & Pelabon (2006), they are on the large side even when we consider that the CV of an area is expected to be twice the CV of the mostly linear measurements in that review. They are in fact similar to the CVs of, the admittedly few cases of, rudimentary traits reported by Hansen *et al.* (2006). Furthermore, the parietal-eye area shows considerably more variation than the area of the parietal scale in which it is embedded. The corresponding CVs for the parietal-scale area were 0.225 ± 0.014, 0.223 ± 0.014, and 0.225 ± 0.014, respectively. By dividing by two, we see that the variation in relative parietal-scale area is comparable to the standard deviations of the logs of head width, head length, and snout–vent lengths, which, at 0.110 ± 0.007, 0.090 ± 0.006, and 0.090 ± 0.006, respectively, were close to the mean CV of 0.113 for quantitative traits reported by Hansen *et al.* (2006).

There was no evidence of a sex difference in relative parietal-eye size. Including sex in the regression of log parietal-eye area on species and log head width gave an estimated difference between male and female log parietal-eye area of 0.016 ± 0.072 (t-test: $t = 0.22$, $P = 0.82$), which corresponds to a 1.6% difference, and regressions on the other size variables gave similar results. Relative parietal-scale area, however, has a tendency to be larger in males (log difference is 0.097 ± 0.050, $t = 1.95$, $P = 0.053$ when regressed on log head width, with similar results obtained for the other variables). Note, however, that the lack of an overall sex effect does not exclude the possibility of sexual dimorphisms in individual species.

AMONG-SPECIES VARIATION

The comparative analyses were based on the estimated species intercepts from the regression of log parietal-eye area on log head width as given in Table 1. We started by investigating whether there are any phylogenetic effects in this variable. Figure 4 shows a support surface for phylogenetic half-life and stationary variance in a model with no predictor variables. The maximum-likelihood estimate has $t_{1/2} = 0.000$, which implies no phylogenetic effects, and the two-unit support set includes half lives up to $t_{1/2} = 0.46$, which is still a moderate effect. The absence of a phylogenetic effect in a variable is, however, not a sufficient reason to avoid phylogenetic comparative analysis because it is still possible that there is a phylogenetic effect in the residuals of a regression of this variable on other variables (Hansen & Orzack 2005; Labra *et al.*, 2009). We therefore checked for phylogenetic inertia in all the models we ran, and, in each case, the maximum-likelihood estimate of $t_{1/2}$ was zero or very close to zero (Table 3). We conclude that relative parietal-eye size is not phylogenetically constrained on this time scale, and this justifies analyzing adaptation with standard nonphylogenetic statistical methods where we also include the eight species for which we lack phylogenetic information. In the remainder, we focus on the results obtained from the nonphylogenetic analysis (still including measurement variance). The phylogenetic analyses are reported in Table 3, and are for the most part consistent with the nonphylogenetic analyses.

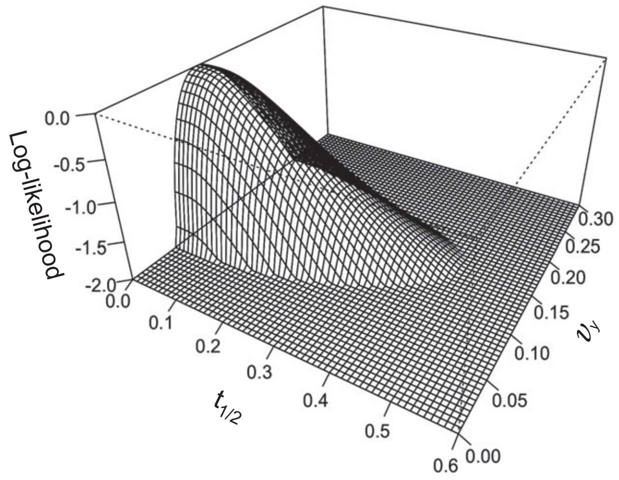

Figure 4. Support surface for phylogenetic half-life and stationary variance in relative parietal-eye area in a model including only an intercept and no predictor variables. The figure shows support (= log likelihood) of models with different values of $t_{1/2}$ and v_y. The flat part of the plot are values that are more than two log-likelihood units worse than the maximum. The best estimate is at $t_{1/2} = 0.000$ and $v_y = 0.106$.

Figure 5A shows the regression of relative parietal-eye area on latitude. The slope is essentially zero and explains less than 1% of the variation, and thus provides no support for the hypothesis of Ralph *et al.* (1979). Similar very weak effects were found for altitude, the thermal index, as well as for the local substrate and operative temperatures (Table 4, Fig. 5B). Among the thermophysiological variables, we found no effect of the selected body temperature (Fig. 5C), although there was an effect from the critical thermal minimum (Fig. 5D, Table 4), which explained 16% of the variance. The estimate indicates that a 1 °C increase in the thermal tolerance to cold

Table 3. Phylogenetic comparative analysis of relative log parietal-eye area in relation to environmental and thermo-physiological predictor variables

Predictor	$t_{1/2}$ (Support region)	v_y	Intercept ± SE	Slope ± SE	R^2	Support	AIC$_c$
None	0.000 (0, 0.461)	0.106	–3.65 ± 0.12	–	–	–8.91	25.15
Latitude	0.026 (0, 0.445)	0.105	–3.54 ± 0.40	–0.004 ± 0.014 °S^{-1}	0.4%	–8.87	28.10
Altitude	0.000 (0, 0.504)	0.106	–3.66 ± 0.17	0.000 ± 0.000 km^{-1}	0.1%	–8.90	28.15
TI	0.015 (0, 0.457)	0.106	–3.63 ± 0.35	–0.000 ± 0.014 °C^{-1}	0.0%	–8.91	28.17
T_e	0.069 (0, 1.106)	0.091	–2.94 ± 0.52	–0.024 ± 0.018 °C^{-1}	8.0%	–8.13	26.61
T_s	0.021 (0, 0.450)	0.106	–3.56 ± 0.67	–0.003 ± 0.022 °C^{-1}	0.1%	–8.90	28.16
T_{sel}	0.048 (0, 0.434)	0.092	–1.46 ± 1.61	–0.069 ± 0.051 °C^{-1}	8.1%	–8.11	26.57
Ct_{min}	0.027 (0, 0.362)	0.100	–3.30 ± 0.35	–0.036 ± 0.035 °C^{-1}	5.2%	–8.14*	26.78*
SVL	0.000 (0, 0.173)	0.055	–4.42 ± 0.35	0.013 ± 0.006 mm^{-1}	25.6%	–5.53	21.42
SVL	0.000 (0, 0.162)	0.046	–4.64 ± 0.71	0.014 ± 0.007 mm^{-1}	27.9%	–4.94*	23.87*
+ Ct_{min}				–0.012 ± 0.040 °C^{-1}			

The first column shows the predictor variable included in the model (TI, thermal index; T_e, operative temperature; T_s, substrate temperature; T_{sel}, selected body temperature; Ct_{min}, critical thermal minimum). The second column shows the best estimate of the phylogenetic half-life, $t_{1/2}$, in units of tree height. The third column shows the stationary variance. The intercept and slope refer to the 'optimal' regression corresponding to the best estimate of $t_{1/2}$ and v_y. The model including snout–vent length (SVL) is the only one with better small-sample corrected Akaike information criterion (AIC$_c$) than the model including only an intercept. Unless otherwise stated, this is based on the twenty-two species in the phylogeny in Fig. 2.

*Not comparable to the other models because data for *Liolaemus schroederi* are missing. The support and AICc for the intercept-only model on the same data are –8.65 and 24.70, respectively.

Table 4. Nonphylogenetic regressions of relative log parietal-eye area on predictor variables

Predictor	Intercept ± SE	Slope ± SE	R^2	Support	AIC$_c$
None	–3.65 ± 0.19	–	–	–12.79	30.04
Latitude	–3.75 ± 0.41	0.004 ± 0.014 °S^{-1}	0.3%	–11.89	30.73
Altitude	–3.77 ± 0.16	0.000 ± 0.000 km^{-1}	3.3%	–11.67	30.30
TI	–3.25 ± 0.31	–0.016 ± 0.012 °C^{-1}	6.9%	–10.99	28.94
T_e	–2.99 ± 0.60	–0.020 ± 0.018 °C^{-1}	5.7%	–10.79*	28.59*
T_s	–4.16 ± 0.70	0.016 ± 0.022 °C^{-1}	2.3%	–10.77*	28.54*
T_{sel}	–2.73 ± 1.97	–0.027 ± 0.057 °C^{-1}	1.0%	–11.21*	29.42*
Ct_{min}	–3.04 ± 0.33	–0.061 ± 0.031 °C^{-1}	15.8%	–8.83†	24.70†
SVL	–4.46 ± 0.34	0.014 ± 0.007 mm^{-1}	23.3%	–9.52	25.99
SVL	–3.91 ± 0.66	0.010 ± 0.007 mm^{-1}	25.0%	–8.04†	25.89†
+ Ct_{min}		–0.034 ± 0.036 °C^{-1}			

The models including thermal index (TI), substrate temperature (T_s), critical thermal minimum (Ct_{min}) and snout–vent length (SVL) have better Akaike information criterion (AIC$_c$) than the intercept-only model. Unless otherwise stated, this is based on 29 species in Table 1 after *L. vallecurensis* is removed.

*Not comparable to the other models because data for *L. maldonadae* are missing. The support and AICc for the intercept-only model on the same data are –12.04 and 28.56, respectively.

†Not comparable to the other models because data for *L. maldonadae* and *L. schroederi* are missing. The support and AICc for the intercept-only model on the same data are –11.74 and 27.98, respectively.

would increase the relative parietal-eye area by about 6%, although approximately half the effect went away when snout–vent length was included in the model. Finally, there was a moderate effect of snout–vent length explaining 23% of the variance (Table 4): An increase of 1 cm in the mean snout–vent length of the species predicts an approximate 14% increase in the relative parietal-eye area of that species. Note here that the correction for size within species does not preclude an additional size effect among species means, and that this effect then comes in addition to the growth-related static allometries within species.

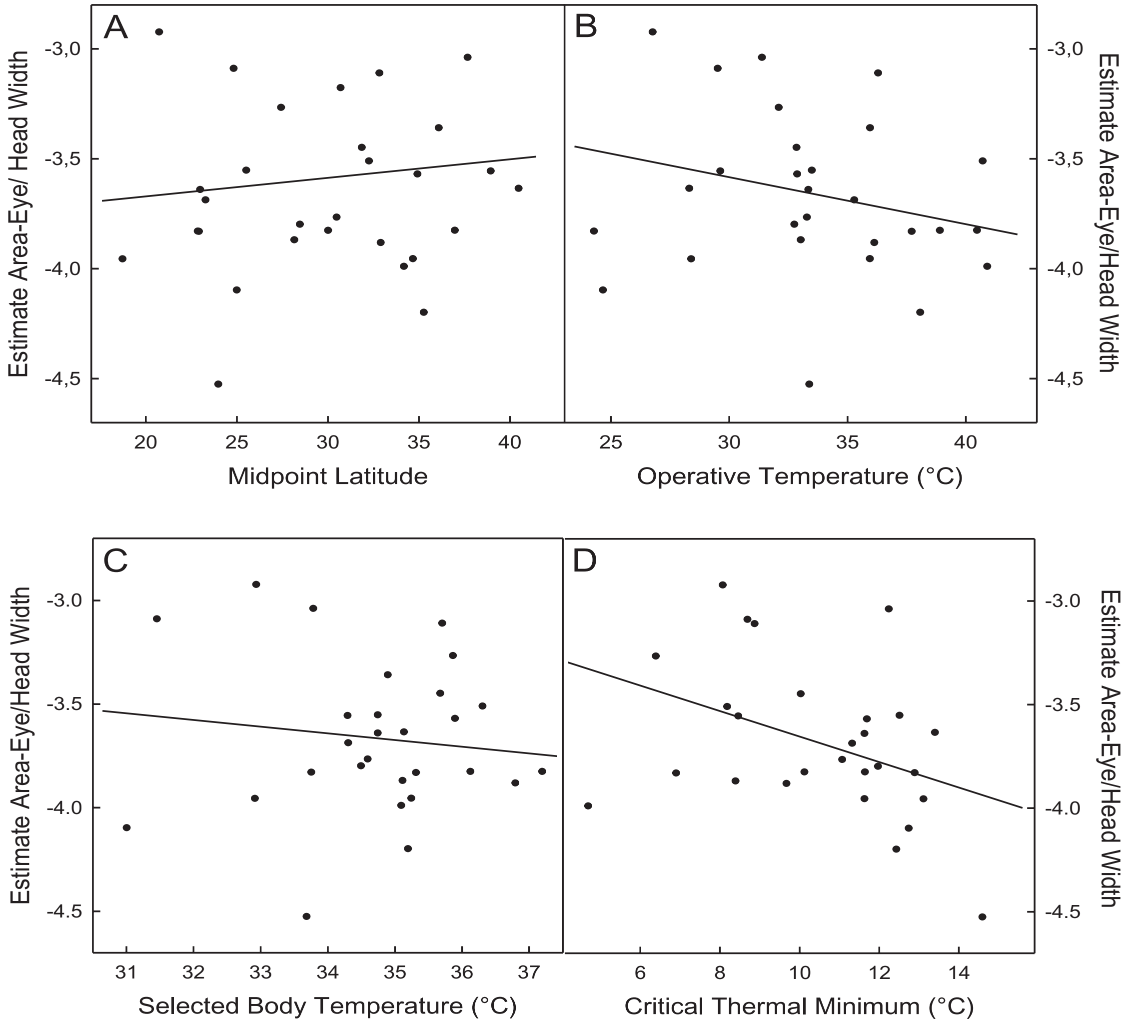

Figure 5. Among-species plots of relative parietal-eye area against selected predictor variables: On the y-axes are the intercepts of log parietal-eye area corrected for log head width (assuming identical slopes within species). A, plot against latitude. B, plot against operative temperature. C, plot against selected body temperature. D, plot against critical thermal minimum. The regression lines are for the nonphylogenetic regressions reported in Table 4.

We repeated all the above analyses with relative parietal-scale area (corrected against head width) as a response variable. The results were similar to those of relative parietal-eye area with no indications of phylogenetic effects or phylogenetic inertia, and only very weak nonsignificant effects of the predictor variables except for snout–vent length and critical thermal minimum, which explained 19.3% and 14.0% of the variance, respectively (other results not shown).

Finally, we plotted relative log parietal-eye area against male and female home-range sizes for five species reported by Fox & Shipman (2003). There was no obvious relationship, although the two species with the smallest home-range sizes also did have the smallest relative parietal-eye sizes (Fig. 6).

DISCUSSION

The results obtained in the present study provide a consistent negative answer to Edinger's (1955) question: the size of the parietal organ is not correlated with the habitat of the reptile, at least not in the case of the genus *Liolaemus* and its climatic habitat. We did not find any evidence for adaptation to any of the ecological variables that we investigated, including latitude, altitude, or any measure of local environmental temperatures. We did see a weak association with thermal tolerance, although some of this may have been the result of a confounding effect of body size. There was no evidence of phylogenetic inertia, and the negative results are therefore unlikely to be a result of phylogenetic constraints. Within species,

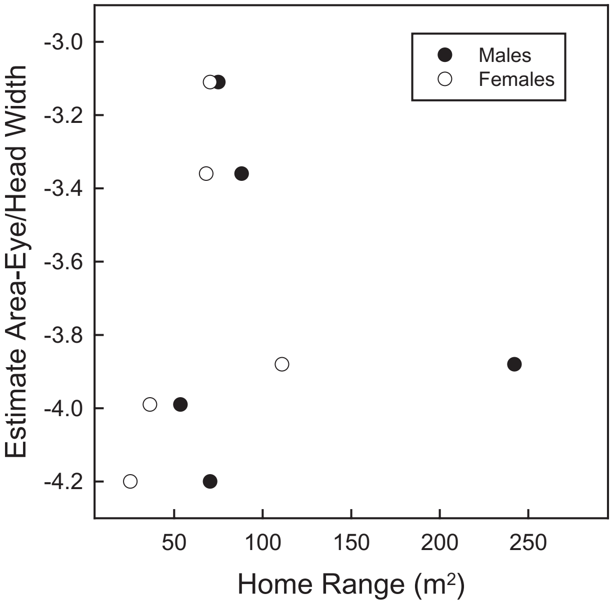

Figure 6. Relative parietal-eye area in relation to home-range size for five species of *Liolaemus*. The home-range sizes taken from Fox & Shipman (2003) are for *L. bellii* (male: 53.5 m^2, female: 36.3 m^2), *L. lemniscatus* (70.3 m^2, 25.2 m^2), *L. monticola* (242.2 m^2, 110.9 m^2), *L. nitidus* (74.9 m^2, 70.3 m^2), and *L. schroederi* (88.1 m^2, 68.1 m^2).

parietal-eye size shows a shallow static allometry, and thus grows more slowly than overall size. After removing the static allometry, however, there was an evolutionary tendency for species with larger bodies to present relatively larger parietal eyes. In addition, there was unusually high variation in parietal-eye size within species.

The results of the present study are not consistent with the macroevolutionary observations of Gundy *et al*. (1975). They found that parietal eyes are more common in lizard taxa that occur at high latitudes, and hypothesized that the absence of a parietal eye was a constraint that made it difficult for lizards to adapt to 'harsher' climates at higher latitude, which may require more accurate reproductive synchrony and/or more accurate thermoregulation (Gundy *et al*., 1975; Ralph, 1975; Gundy & Wurst, 1976a, b; Ralph *et al*., 1979). This hypothesis would entail that the third eye would be maintained by relatively strong selection at higher latitudes, although not at lower latitudes. If this was true for *Liolaemus*, we might expect larger parietal eyes at higher latitudes and also low variation within species, which we did not find, although it is possible that the parietal eye has some simple on/off function, which may depend on its presence but not on its exact size above some minimum, and we cannot rule out that the latitudinal range we explored did not extend into areas where the parietal eye may become unimportant.

Likewise, the results of the present study do not support the hypotheses that the parietal eye acts as a dawn–dusk detector (Solessio & Engbretson, 1993) or as a season detector (Quay, 1979). Arguably, the dawn–dusk hypothesis would predict a larger eye at higher latitudes where the transitions from dawn to dusk are more gradual and change more with season. The season-detector hypothesis may also predict a relation of eye size to latitude and altitude, although one may question whether a larger parietal eye would be expected where seasonality is pronounced and therefore important, or faint and therefore hard to detect.

One of the most consistent roles of the pineal complex as a whole is in the regulation of body temperature (Ralph *et al*., 1979). It is thus a reasonable hypothesis that the parietal eye of tetrapods may have had its primitive function in relating thermoregulation to photic stimuli. Lizards are the most active and fine-tuned behavioural thermoregulators among extant ectothermic vertebrates, and it may then not be a coincidence that the most developed parietal eyes are found in this group (along with the tuatara, *Sphenodon*). These two facts suggest that a relatively complex parietal eye may be adaptively maintained in many lizard species as a photic sensory organ that helps the animal achieve fine-tuned behavioural thermoregulation. This is also supported by the observation that ablation or occlusion of the parietal eye tends to make lizards bask more and achieve higher body temperatures (Eakin, 1973; Quay, 1979; Ralph *et al*., 1979; Tosini, 1997). Although the results of the present study did not support the hypothesis that parietal-eye size is adapted to major climatic axes such as latitude, altitude, and temperature, this does not rule out an involvement in thermoregulation, which after all is an important aspect of life for all our study species (Labra *et al*., 2008). Tentatively, we found an indication that the size of the parietal eye may be adapted to the critical thermal minimum of the species, or more likely, to ecological or physiological variables related to the critical thermal minimum. The critical thermal minimum itself is not strongly related to any ecological variable we have investigated, although it does have a moderately strong negative relation to snout–vent length (Labra *et al*., 2009) and because including snout–vent length in the analysis removed much of the effect, we are left with little evidence for adaptation of parietal-eye size to thermal tolerance.

In general, a failure to find support for specific adaptive hypotheses does not mean that the trait in question is unaffected by selection. Quantitative characters, such as the area of an eye, are typically highly evolvable and mutable (Houle, Morikawa & Lynch, 1996) and, in the absence of selection, they are expected to change rapidly as a result of mutation and genetic drift (Lynch, 1990). The within-one-order-of-magnitude range of variation of parietal-eye area in *Liolaemus* shows that there exists some form of constraint on evolution, whether this is the result of direct stabilizing selection caused by some function of the eye, or the result of indirect selection caused by pleiotropic constraints. Our data are consistent, however, with relatively weak constraints on parietal-eye size. The absence of phylogenetic effects may be consistent not only with rapid adaptation, but also with rapid, random evolutionary changes within a limited size range. The relatively large coefficients of variation and the fact that the parietal eye has considerably more variation, both within and among species, than the parietal scale in which it is embedded also indicate that there is no strong uniform selection for accurate size of the parietal eye.

Alternatively, the within-species variation in parietal-eye size may be caused by plasticity in relation to some unknown variables. We showed that there is little variation in relation to sex, although we cannot rule out the possibility that there is plasticity in relation to other unmeasured variables. Some of the variation in parietal-eye area is undoubtedly a result of measurement error, which we have not quantified, although it is unlikely that measurement error could explain the very large difference in variation between the parietal eye and the parietal scale.

Unfortunately, there is only fragmentary knowledge about the ecology, life-history, and social behaviour of *Liolaemus* (Labra, 2008). It was therefore not possible to construct a convincing test of what is perhaps the best-established functional hypothesis for the lizard parietal eye, namely that it is an organ of orientation, specifically a sun-compass (Adler & Phillips, 1985; Ellis-Quinn & Simon, 1991; Freake, 1999, 2001; Foa *et al*., 2009; Beltrami *et al*., 2010). First of all, this hypothesis predicts that the quantitative size of the parietal eye should be important, and is thus to some extent contradicted by our finding of high variation. Most species of *Liolaemus* are sit and wait foragers (Cooper, 1995); it is thus possible that they may be relatively stationary compared to other lizards, and the parietal eye may be less important in this group. This can be settled by similar quantitative investigations in other groups. Second, the sun-compass hypothesis predicts that parietal-eye size should relate to how much the species move, to their habitat, and perhaps to factors such as cloudiness, tree cover, and the presence of alternative means of orientation. We were only able to present a highly preliminary relation of parietal-eye size to home-range size, and the result is inconclusive, albeit not inconsistent with a positive effect (Fig. 6). A third possible prediction from the sun-compass hypothesis is that the parietal eye may have its most important function during dispersal, which again predicts that sex- or age-related differences may evolve. We did not find sex-related differences, although we did not have a good representation of both sexes for many of the species, and it would have been premature to test more refined hypotheses about, for example, the relation of possible species-specific sexual dimorphisms to ecological variables. We also do not have data for juvenile individuals, and there is no information about dispersal in *Liolaemus*. The shallow static allometry is, however, consistent with a primary function at the juvenile stage.

An important caveat is that the area of the ‘cornea’ is the ecologically relevant variable. We have no direct evidence that the size of the parietal eye itself is correlated to the measured area of the transparent skin above it. This is reminiscent of an old debate as to whether the size of the parietal eye is correlated with the size of the parietal foramen in which it is embedded. Edinger (1955) concluded that it was. In any case, the area of the transparent ‘cornea’ is likely to be a functionally significant variable regardless of its exact relation to the size of the underlying eye because it determines how much light can reach the eye. There is a complication of this issue, however, in that the functional area of the cornea is sometimes reduced by some form of plaque or calcification.

In conclusion, we have shown that there is little relation of the size of the parietal eye to latitude or related climatic variables in *Liolaemus*, and we found little evidence for a relation to thermophysiological variables. The results obtained in the present study are consistent with weakly selected or qualitative (e.g. threshold) functions of the parietal eye in this group. At present, there is insufficient data for a proper comparative test of the sun-compass hypothesis and other ecobehavioural hypotheses in *Liolaemus*.

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