

Head shape evolution in Gymnophthalmidae: does habitat use constrain the evolution of cranial design in fossorial lizards?

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Abstract

Habitat usage comprises interactions between ecological parameters and organismal capacities, and the selective pressures that ultimately determine the outcome of such processes in an evolutionary scale may be conflicting when the same morphological structure is recruited for different activities. Here, we investigate the roles of diet and locomotion in the evolution of cranial design in gymnophthalmid lizards and test the hypothesis that microhabitat use drives head shape evolution, particularly in head-first burrowers. Morphological factors were analysed in relation to continuous ecological indexes (prey hardness and substrate compactness) using conventional and phylogenetic approaches. Results suggest that the evolution of head morphology in Gymnophthalmidae was shaped under the influence of microhabitat use rather than diet: burrowers have shorter heads with lower rostral angulation, independently of the prey consumed. Food preferences appear to be relatively conserved throughout the phylogeny of the group, which may have permitted the extensive radiation of gymnophthalmids into fossorial microhabitats.

Introduction

The phenotypic variation observed in natural lineages results from evolutionary processes that likely reflect interactions among selective pressures inherent to the environment where the populations evolved (Pianka, 1973; Losos *et al.*, 1997; Vitt *et al.*, 2003; Lamb & Bauer, 2006). In this context, the selection of a given environment by an organism is directly influenced by the interaction between its morphological and physiological capacities and ecological factors such as climate, structural habitat complexity or food availability (Pianka, 1973; Pounds, 1988; Huey, 1991; Vitt *et al.*, 2003). The selective pressures that ultimately determine the outcome of such evolutionary processes, however, may be very distinct, and the same morphological structures can be recruited for different activities. Because an organism may not be able to optimize all functions simultaneously, the morphological product of evolutionary processes

likely reflects the conflicting selective pressures that result in functional trade-offs (Herrel *et al.*, 2007, 2009).

The cranium is an ideal example of a complex functional integrated system that plays an essential role in a variety of activities, including defensive and sexual behaviour, locomotion, prey capture and ingestion (for example in lepidosaurs, see Cooper & Vitt, 1993; Schwenk, 2000; Herrel *et al.*, 2001a, 2007; Lappin & Husak, 2005; Kohlsdorf *et al.*, 2008). In this context, the cranial system likely evolves under a scenario involving functional trade-offs. For example, the type of prey selected possibly has a strong effect on the evolution of head size, because enhanced bite performance allows predators to effectively crush hard prey (Herrel *et al.*, 2002a,b, 2007; Vanhooydonck *et al.*, 2007). Bite performance can be altered through changes in cranial structure, such as increases in head width and height, important to accommodate larger jaw muscles, and increments in the jaw closing in-lever length, all of which improve bite forces (Herrel *et al.*, 2001b,c; Vanhooydonck *et al.*, 2007). However, a powerful bite may compromise the accuracy and speed needed during prey capture, as fast jaw closing is benefited by longer

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snouts and jaw out-levers, as well as a longer in-lever for jaw opening (Herrel *et al.*, 2001b,c; Vanhooydonck *et al.*, 2007). The evolution of the cranial system therefore clearly illustrates how functional trade-offs may affect phenotypic evolution (Harmon *et al.*, 2005; Kohlsdorf *et al.*, 2008; Herrel *et al.*, 2009).

The functional trade-offs that affect the evolution of cranial morphology are not necessarily restricted to diet but, instead, may also involve selective pressures that are related, for example, to locomotion in different microhabitats. Locomotor performance affects fitness (Aerts *et al.*, 2000) and is a key factor in predator escape, foraging and territory defence (Huey & Dunham, 1987; Hertz *et al.*, 1988; Garland *et al.*, 1990; Garland & Losos, 1994; Kohlsdorf *et al.*, 2004). The relationship between head morphology and locomotion may differ among species that move in different microhabitats, as the effects of head shape and size on locomotor performance may differ, for example, between animals that climb vertical structures (e.g. Vanhooydonck & Van Damme, 1999; Herrel *et al.*, 2001a) and fossorial animals (e.g. Gans, 1969, 1975; Teodecki *et al.*, 1998; De Schepper *et al.*, 2005; Herrel & Measey, 2010).

Despite the predicted effects of diet and locomotion on the evolution of the cranium, the outcome from possible conflicting selective pressures acting on head shape is rarely investigated (but see De Schepper *et al.*, 2005; Kohlsdorf *et al.*, 2008; Vanhooydonck *et al.*, 2011). Thus, it remains unclear whether selective pressures imposed by dietary niche override selection on the cranial system imposed by locomotion in different microhabitats in the evolution of head shape in vertebrates. This issue is particularly pertinent in squamates, as most studies suggest that head shape evolves under selective pressures for biting (in either a sexual or natural selection context). Yet some studies have suggested that selection on head shape may be driven by the demands for locomotion rather than biting, especially in fossorial taxa where constraints on head shape evolution by the medium are likely stringent (Vanhooydonck *et al.*, 2011). For example, amphisbaenas have short skulls and are efficient burrowers, but bite weakly (Gans, 1969). In head-first burrowers, therefore, head shape likely evolves under constraints for efficient soil penetration rather than selection for biting.

The discernment of the role of burrowing in head shape evolution might be particularly favoured by studies investigating cranial evolution in clades that present both fossorial and epigeal representatives. In this context, the lizard family Gymnophthalmidae is an ideal model system as it includes both epigeal lizard-like species and elongated-limbed burrowers (e.g. MacLean, 1974; Presh, 1980; Pellegrino *et al.*, 2001; Kohlsdorf & Wagner, 2006; Kohlsdorf *et al.*, 2010). These species are distributed within a wide range of South American microhabitats (Presh, 1980; Ávila-Pires, 1995; Rodrigues, 1996), and an extensive ecological literature is available. In this study, we analyse head morphology in

gymnophthalmid lizards and test the hypothesis that microhabitat use drives the evolution of the cranial design, especially in head-first burrowers. Specifically, we expect that species that occupy microhabitats which offer greater resistance to displacement have more streamlined heads to facilitate soil penetration, whereas those that move on the surface and prey on hard food items should have relatively more robust heads and longer jaw closing in-levers, independent of the microhabitat they occupy.

Material and methods

Animals and morphometric traits

This study was conducted using 48 lizard species of Gymnophthalmidae that were available in the Herpetological Collection of the Museum of Zoology from the University of São Paulo (MZUSP), in Brazil. Two to 20 individuals from the same population or area (in order to reduce possible effects of intraspecific variation) were measured for each species. Both adult males and females were included here due to the limited amount of representatives for some gymnophthalmid species in the collection of MZUSP. The sex of each specimen was inferred by the presence of femoral pores (which in males are located ventrally at the cloaca and on upper hind limbs) or by the reverted hemipenes.

Nine morphometric traits were obtained for each individual: (i) trunk length (measured from the neck to the cloaca), (ii) head length (measured from the posterior extremity of the parietal scale to the tip of the snout), (iii) head width (given by the largest distance between the temporal scales), (iv) head height (given by the maximum distance between the base of the mandible and the parietal surface), (v) nostril height (given by the largest distance between the base of the jaw and the nasal scale), (vi) nostril distance (given by the maximum distance between the nostrils), (vii) lower jaw length (measured from the back of the retroarticular process to the tip of the lower jaw), (viii) quadrate-tip (measured from the back of the quadrate bone to the tip of the upper jaw) and (ix) snout length (measured from the back of the jugal bone to the tip of the upper jaw). From these measurements, four additional morphological variables were calculated, based on biomechanical predictions: (x) rostral lateral decline (given by the ratio between head width and nostrils distance), (xi) rostral angulation (given by ratio between head height and nostril height), (xii) open in-lever (being the subtraction of quadrate-tip length from lower jaw length; Metzger and Herrel, 2005; Kohlsdorf *et al.*, 2008) and (xiii) and close in-lever (being the quadrate-tip minus the snout length; Metzger and Herrel, 2005; Kohlsdorf *et al.*, 2008). All measures were made by FCB, using a digital calliper to the nearest 0.01 mm (Mitutoyo Inc., Mississauga, Canada); variables used in the statistical analyses are illustrated in Fig. 1.

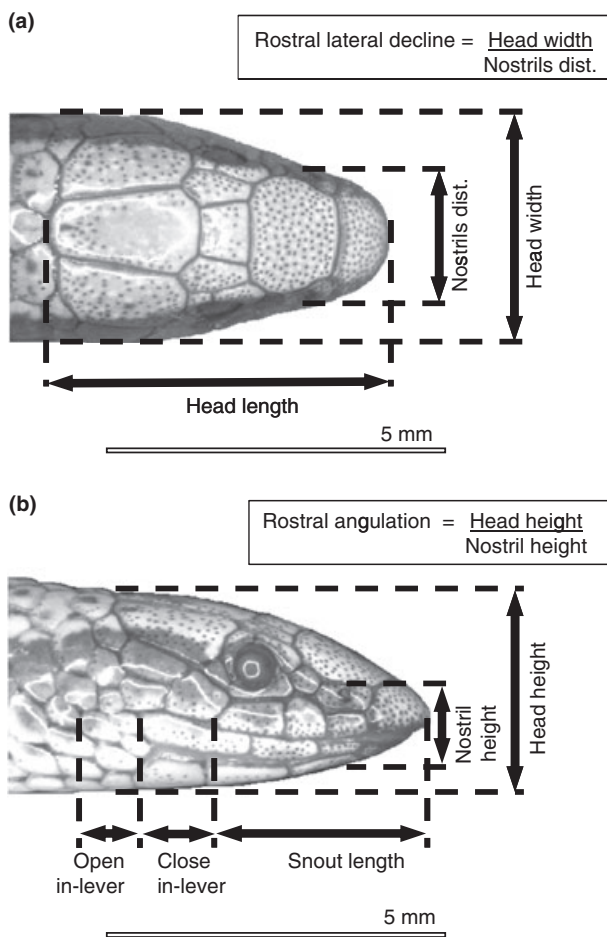


Fig. 1 Morphometric variables considered in the statistical analyses, from a dorsal (a) and a lateral (b) perspective. The species given for reference is *Nothobachia ablephara*.

Microhabitat and diet

Two ecological indexes were assembled, one based on microhabitat and the other on diet, and were used to investigate possible evolutionary relationships between head shape and ecology in gymnophthalmid lizards. The literature used to estimate these indexes is detailed in the Supporting information. Microhabitat usage was treated as a continuous variable (Table 1), based on an index (MI) calculated according to the following equation:

$$MI = \sum (\beta * F_R)$$

In this equation, β and F_R represent, respectively, the proportion of each microhabitat used by a given species and the force of resistance (which was assessed proportionally from average forces of resistance – $A_{Fresist}$ – empirically estimated, as detailed below and in the Supporting information) of each substrate to head

displacement. MI ranges from 0 to 1, with species that have values closer to 1.0 being ones that occupy microhabitats that offer the largest resistance to displacement. We inferred β based on the proportion of microhabitat use for each species, according to published data. Articles in ecology often present data of how many individuals from a given species were observed in each substrate; based on the total number of individuals mentioned in one or more articles, we have calculated the percentages corresponding to each microhabitats. For example, a species would have $\beta = 1.0$ for ground if all individuals reported were observed/collected under the surface, whereas other species might have $\beta = 0.4$ for grass and $\beta = 0.6$ for bush if 40% of the individuals were reported in the former substrate and 60% of the individuals were reported in the later microhabitat. The literature available from ecological studies comprises several microhabitat categories; from those, we established eight general classes of substrates used by gymnophthalmid lizards: (i) ground (fossorial animals found under the surface, in sandy or rooted soils); (ii) leaf-litter (individuals observed in the leaf-litter, or under bushes and bromeliads in the ground); (iii) shelter (animals observed inside orifices that were already available, as in the holes or under fallen logs, inside termite nests or in rock crevices); (iv) grass (individuals in open fields and other areas covered by grasses); (v) surface (animals observed at open ground or on top of rocks and trunks); (vi) bush (arboreal individuals observed on branches, leaves or bushes); (vii) mud (semi-aquatic animals moving in streams and swamps); and (viii) water (individuals swimming during prey capture or predator escape).

The substrate resistance to head displacement (F_R) was measured by adding weight hanging freely under an angle of 90° to a lead model (with shape similar to a lizard head) burrowed into a given substrate and quantifying the minimal weight necessary to horizontally displace the model into each substrate/microhabitat category in an acrylic box. ‘Surface’ and ‘bush’ were assumed to have an ‘air resistance’, which would be equal to zero. For all other substrate/microhabitat categories, we repeated the tests five times and obtained the average force of resistance ($A_{Fresist}$). In the case of ‘shelter’, we used a narrow piece of wood to simulate the friction of the head (lead model) with the substrate. For ‘ground’ and ‘leaf-litter’, the lead model was buried into a depth of 1 cm before the tests; ‘leaf-litter’ consisted of a layer of dry loose leaves with small grasses and ‘ground’ consisted of sand substrate; both were transported to the laboratory from sites where we collected gymnophthalmid lizards. Average resistance forces for each substrate were assessed proportionally on a variable F_R , which in theory could range from 1.00 (substrates that impose the largest resistance to head displacement) to 0.00 (microhabitats with lowest resistance, which would be equal to ‘air resistance’), as detailed in the Supporting information.

Table 1 Morphological traits (means \pm standard errors, all in mm) and ecological indexes for gymnophthalmid lizards.

Species	<i>n</i>	Trunk length	Head length	Snout length	Open in-lever	Close in-lever	Rostral lateral decline	Rostral angulation	DI	MI
<i>Neusticurus ecleopus</i>	15	44.07 \pm 0.94	11.66 \pm 0.29	4.95 \pm 0.12	3.76 \pm 0.15	4.49 \pm 0.22	2.41 \pm 0.06	3.39 \pm 0.07	0.401	0.407
<i>Ptychoglossus brevifrontalis</i>	4	37.43 \pm 2.32	8.75 \pm 0.41	3.48 \pm 0.19	3.33 \pm 0.31	3.29 \pm 0.22	1.84 \pm 0.08	2.19 \pm 0.07		0.494
<i>Pantodactylus schreibersii schreibersii</i>	20	26.81 \pm 0.52	6.99 \pm 0.14	2.86 \pm 0.05	1.69 \pm 0.05	2.72 \pm 0.06	1.82 \pm 0.03	2.09 \pm 0.03	0.255	0.352
<i>Prionodactylus oshaughnessyi</i>	20	34.57 \pm 0.33	9.64 \pm 0.11	4.19 \pm 0.05	2.62 \pm 0.10	3.75 \pm 0.07	2.25 \pm 0.05	2.73 \pm 0.04	0.285	0.436
<i>Prionodactylus argulus</i>	4	25.36 \pm 2.14	7.48 \pm 0.32	3.39 \pm 0.23	1.67 \pm 0.17	3.27 \pm 0.21	2.04 \pm 0.07	3.04 \pm 0.17	0.213	0.112
<i>Cercosaura ocellata ocellata</i>	20	34.79 \pm 0.54	9.32 \pm 0.14	3.89 \pm 0.05	2.02 \pm 0.07	3.90 \pm 0.06	1.78 \pm 0.02	2.37 \pm 0.03	0.339	0.466
<i>Prionodactylus eigenmanni</i>	20	31.20 \pm 0.67	9.33 \pm 0.15	4.01 \pm 0.06	2.45 \pm 0.09	3.52 \pm 0.11	2.10 \pm 0.04	2.63 \pm 0.03	0.205	0.588
<i>Pantodactylus quadrilineatus</i>	20	29.03 \pm 0.64	7.44 \pm 0.10	3.12 \pm 0.05	1.90 \pm 0.09	2.77 \pm 0.07	1.86 \pm 0.04	2.33 \pm 0.03		0.672
<i>Neusticurus juruazensis</i>	2	39.50 \pm 0.06	10.24 \pm 0.37	4.30 \pm 0.09	3.13 \pm 0.06	3.90 \pm 0.14	2.01 \pm 0.02	3.45 \pm 0.19	0.388	0.603
<i>Neusticurus bicarinatus</i>	18	69.70 \pm 1.63	17.38 \pm 0.43	8.05 \pm 0.19	4.54 \pm 0.22	7.83 \pm 0.27	2.50 \pm 0.07	3.57 \pm 0.07	0.451	0.293
<i>Neusticurus rudis</i>	7	39.29 \pm 3.45	11.92 \pm 0.32	5.20 \pm 0.27	3.54 \pm 0.28	4.38 \pm 0.24	2.14 \pm 0.06	3.58 \pm 0.07	0.781	0.182
<i>Placossoma cordylinum</i>	17	30.79 \pm 1.46	8.77 \pm 0.33	4.20 \pm 0.18	2.55 \pm 0.14	3.02 \pm 0.11	2.31 \pm 0.06	2.83 \pm 0.05		0.504
<i>Placossoma glabellum</i>	19	38.71 \pm 1.22	9.95 \pm 0.22	4.58 \pm 0.10	2.79 \pm 0.11	3.58 \pm 0.12	2.28 \pm 0.05	2.71 \pm 0.05		0.504
<i>Bachia dorbigny</i>	5	59.20 \pm 2.49	6.31 \pm 0.19	2.32 \pm 0.05	1.59 \pm 0.12	2.04 \pm 0.13	1.59 \pm 0.03	2.01 \pm 0.04		0.569
<i>Bachia monodactyla monodactyla</i>	9	54.97 \pm 1.18	6.52 \pm 0.11	2.58 \pm 0.03	1.34 \pm 0.06	1.94 \pm 0.09	1.87 \pm 0.04	2.30 \pm 0.05		0.569
<i>Bachia flavescens</i>	5	54.36 \pm 5.52	6.68 \pm 0.54	2.58 \pm 0.19	1.54 \pm 0.29	1.95 \pm 0.15	2.02 \pm 0.09	2.23 \pm 0.15	0.339	0.662
<i>Bachia panoplia</i>	15	60.26 \pm 1.91	7.78 \pm 0.13	2.79 \pm 0.06	1.96 \pm 0.11	2.82 \pm 0.07	1.71 \pm 0.03	1.95 \pm 0.04		0.918
<i>Bachia bresslaui</i>	8	69.57 \pm 4.36	8.40 \pm 0.30	3.31 \pm 0.16	2.11 \pm 0.04	2.68 \pm 0.12	1.80 \pm 0.03	2.01 \pm 0.03	0.348	0.959
<i>Colobosauroides cearensis</i>	1	29.70 \pm 0.00	6.35 \pm 0.00	2.45 \pm 0.00	1.94 \pm 0.00	2.46 \pm 0.00	1.66 \pm 0.00	2.14 \pm 0.00		0.672
<i>Anotosaura vanzolinia</i>	18	30.51 \pm 0.73	5.33 \pm 0.08	2.05 \pm 0.04	1.59 \pm 0.07	1.74 \pm 0.04	1.74 \pm 0.03	1.86 \pm 0.02		0.628
<i>Leposoma percarinatum</i>	20	25.40 \pm 0.37	6.55 \pm 0.07	2.62 \pm 0.04	1.60 \pm 0.09	2.93 \pm 0.08	1.84 \pm 0.03	2.13 \pm 0.03	0.199	0.528
<i>Leposoma guianense</i>	20	24.21 \pm 0.38	6.66 \pm 0.08	2.54 \pm 0.04	1.26 \pm 0.04	3.00 \pm 0.06	2.01 \pm 0.04	2.24 \pm 0.03	0.188	0.538
<i>Leposoma scincoides</i>	20	30.48 \pm 0.61	7.95 \pm 0.11	3.05 \pm 0.03	2.22 \pm 0.08	3.29 \pm 0.07	2.06 \pm 0.04	2.26 \pm 0.03	0.242	0.672
<i>Arthrosaura kockii</i>	5	31.15 \pm 1.86	9.11 \pm 0.33	3.76 \pm 0.11	2.30 \pm 0.12	3.55 \pm 0.22	1.94 \pm 0.07	2.21 \pm 0.08	0.170	0.280
<i>Ecpleopus gaudichaudi</i>	20	29.07 \pm 0.47	6.22 \pm 0.08	2.28 \pm 0.04	1.97 \pm 0.09	2.34 \pm 0.05	1.74 \pm 0.03	2.09 \pm 0.03	0.178	0.619
<i>Arthrosaura reticulata</i>	15	40.25 \pm 1.30	10.18 \pm 0.26	3.90 \pm 0.12	3.31 \pm 0.19	4.28 \pm 0.09	1.80 \pm 0.04	2.44 \pm 0.04	0.298	0.589
<i>Micrablepharus maximiliani</i>	20	29.45 \pm 0.55	6.56 \pm 0.07	3.12 \pm 0.04	1.95 \pm 0.06	2.58 \pm 0.07	1.87 \pm 0.03	1.86 \pm 0.02	0.221	0.356
<i>Micrablepharus atticolus</i>	20	27.78 \pm 0.48	6.14 \pm 0.07	2.88 \pm 0.03	1.66 \pm 0.06	2.54 \pm 0.04	1.82 \pm 0.02	1.85 \pm 0.03	0.274	0.395
<i>Tretioscincus agilis</i>	2	41.59 \pm 4.19	9.23 \pm 0.73	4.44 \pm 0.36	3.05 \pm 0.28	3.95 \pm 0.53	2.03 \pm 0.08	1.92 \pm 0.13	0.383	0.083
<i>Tretioscincus oriximinensis</i>	20	31.64 \pm 0.48	7.09 \pm 0.10	3.42 \pm 0.06	1.99 \pm 0.06	2.96 \pm 0.06	1.85 \pm 0.02	2.07 \pm 0.02	0.192	0.333
<i>Procellosaurinus erythrocerus</i>	20	21.18 \pm 0.68	4.77 \pm 0.05	2.13 \pm 0.03	1.56 \pm 0.03	1.88 \pm 0.05	1.83 \pm 0.03	1.92 \pm 0.03	0.182	0.795
<i>Procellosaurinus tetradactylus</i>	7	21.57 \pm 0.91	5.01 \pm 0.05	2.17 \pm 0.05	1.57 \pm 0.07	1.98 \pm 0.05	1.82 \pm 0.06	1.78 \pm 0.05		0.836
<i>Vanzosaura rubricauda</i>	20	25.17 \pm 0.39	5.59 \pm 0.07	2.45 \pm 0.03	1.94 \pm 0.05	2.01 \pm 0.03	2.02 \pm 0.03	2.24 \pm 0.03	0.218	0.308
<i>Nothobachia ablephara</i>	17	48.40 \pm 0.77	5.73 \pm 0.06	2.58 \pm 0.03	1.65 \pm 0.05	1.65 \pm 0.04	1.84 \pm 0.03	1.83 \pm 0.03	0.127	0.891
<i>Calyptommatius leioplepis</i>	20	52.50 \pm 0.86	6.02 \pm 0.07	2.89 \pm 0.04	2.06 \pm 0.05	1.26 \pm 0.03	2.42 \pm 0.03	1.90 \pm 0.02	0.200	1.000
<i>Calyptommatius nicterus</i>	20	58.13 \pm 0.79	6.07 \pm 0.06	2.91 \pm 0.04	1.62 \pm 0.04	1.48 \pm 0.04	2.08 \pm 0.03	1.82 \pm 0.02	0.174	1.000
<i>Calyptommatius sinebrachiatus</i>	20	51.69 \pm 0.69	5.82 \pm 0.06	2.90 \pm 0.04	1.84 \pm 0.05	1.26 \pm 0.04	2.04 \pm 0.03	1.83 \pm 0.02	0.138	1.000
<i>Gymnophthalmus underwoodi</i>	3	28.46 \pm 2.25	5.50 \pm 0.39	2.61 \pm 0.19	1.60 \pm 0.02	1.87 \pm 0.14	1.85 \pm 0.09	2.00 \pm 0.04	0.175	0.409
<i>Gymnophthalmus vanzoi</i>	1	26.15 \pm 0.00	5.75 \pm 0.00	2.42 \pm 0.00	1.76 \pm 0.00	1.90 \pm 0.00	1.60 \pm 0.00	2.10 \pm 0.00		0.672
<i>Psilophthalmus paeminus</i>	10	22.67 \pm 0.93	4.49 \pm 0.08	1.93 \pm 0.05	1.61 \pm 0.04	1.66 \pm 0.02	2.01 \pm 0.05	1.89 \pm 0.02		0.754
<i>Colobosaura modesta</i>	20	37.44 \pm 0.55	8.69 \pm 0.13	3.42 \pm 0.05	2.88 \pm 0.08	3.24 \pm 0.05	1.86 \pm 0.03	1.92 \pm 0.03	0.213	0.422
<i>Colobosaura mentalis</i>	4	42.92 \pm 3.05	10.23 \pm 0.48	4.30 \pm 0.17	3.12 \pm 0.07	3.43 \pm 0.27	1.74 \pm 0.05	1.86 \pm 0.02		0.672
<i>Iphisa elegans</i>	14	39.63 \pm 0.90	8.58 \pm 0.12	3.21 \pm 0.03	2.95 \pm 0.07	3.60 \pm 0.10	1.79 \pm 0.02	1.75 \pm 0.02	0.241	0.592
<i>Heterodactylus imbricatus</i>	10	87.34 \pm 1.66	13.01 \pm 0.26	5.61 \pm 0.09	4.07 \pm 0.20	5.90 \pm 0.20	1.67 \pm 0.02	1.81 \pm 0.06		0.672
<i>Colobodactylus taunayi</i>	14	44.35 \pm 1.18	8.20 \pm 0.15	3.49 \pm 0.10	2.97 \pm 0.08	3.44 \pm 0.09	1.67 \pm 0.03	1.78 \pm 0.03		0.672
<i>Rachisaurus brachylepis</i>	1	48.97 \pm 0.00	8.97 \pm 0.00	3.69 \pm 0.00	2.82 \pm 0.00	2.65 \pm 0.00	1.59 \pm 0.00	2.31 \pm 0.00		1.000
<i>Alopoglossus carinicaudatus</i>	17	41.80 \pm 0.70	10.70 \pm 0.14	4.15 \pm 0.07	3.53 \pm 0.12	4.43 \pm 0.04	1.99 \pm 0.04	2.17 \pm 0.02	0.220	0.541
<i>Alopoglossus atriventris</i>	15	32.47 \pm 0.67	9.65 \pm 0.15	3.90 \pm 0.07	2.96 \pm 0.10	3.92 \pm 0.06	2.05 \pm 0.03	2.20 \pm 0.04	0.173	0.500

n, numbers of specimens measured; DI, diet index; MI, microhabitat index.

The other ecological index included here was diet, which was also treated as a continuum and calculated as an index (DI) according to the following equation:

$$DI = \sum (\alpha * F_C)$$

In this equation, α and F_C represent, respectively, the percentage of prey types consumed by a given species and

the average force necessary for crushing that prey category (soft, intermediate or hard). DI ranges from 0.1 to 1, with species showing values close to 1.0 being those that have large proportions of hard food items in their diet. Data on diet were recovered from the literature but were available for only 32 species of Gymnophthalmidae (in contrast to microhabitat use, which was assessed for the 48 species measured). The proportions of prey consumed by each species were used to calculate the value of α , according to the total number of stomachs studied that was available in the literature. Prey types were classified into functional groups according to Borror & De Long (1988) and Vanhooydonck *et al.* (2007): (i) soft-bodied prey – Isoptera, Diptera, Dermaptera, Psocoptera, Thysanura, Thysanoptera, larvae and eggs of invertebrates, Collembola, Arachnida, Anellida, Platyhelmintha and Nematelmintha; (ii) intermediate prey – Blattaria, Mantidae, Phasmidae, Homoptera, Myriapoda, Isopoda and Mollusca; and (iii) hard prey – Hemiptera, Coleoptera, Hymenoptera, Formicidae, Decapoda, plants and vertebrates. The average force ($A_{F_{Crush}}$, in Newtons) required to crush different prey types was inferred based on the studies that examined the relationship between bite force and the hardness of some food items (Herrel *et al.*, 1999, 2001b; Aguirre *et al.*, 2003). The average forces for crushing each prey type were assessed proportionally on a variable F_C , as detailed earlier for the microhabitat index (see Supporting information for more details).

The ecological indexes used in this study were calculated based on the proportions of microhabitat use and prey type ingested by individuals of a given species, but they might be biased by variation among populations. Whenever it was possible, we tried to measure a single population for each species studied and pursued those for which we had published data available (for diet and microhabitat use) or knew it was ecologically similar to ones described in the literature. About 38% of the populations measured corresponded to the same population for which published information of microhabitat and diet was available, and another 62% of the populations measured were at least from the same Brazilian biome. Detailed tables with ecological information are presented in the Supporting information.

Statistics

Seven morphometric variables, based on the nine measurements taken, were used in the statistical analyses: trunk length, head length, snout length, open in-lever; close in-lever, rostral lateral decline and rostral angulation (Table 1). All mean values were \log_{10} -transformed. The variables head length, snout length, open in-lever and close in-lever were regressed against trunk length and the residuals were used in the analyses, in order to remove the effects of body size. The randomization test described by Blomberg *et al.* (2003) was used to test for the presence of significant phylogenetic signal in the

variables analysed, using the module 'PHYSIG' in MATLAB (v. R2008a; The MathWorks Inc., Natick, MA, USA).

The associations between ecological factors and head shape in Gymnophthalmidae were tested based on the regressions performed with principal components composed of morphometric variables. Factor analyses coupled to a varimax rotation were performed on the seven morphological traits studied using SPSS (v.16.0 for PC; SPSS Inc., Chicago, IL, USA), in order to explore head shape variation in Gymnophthalmidae. Components with eigenvalues > 1 were retained, and their PC scores were saved and then used as input for regression models with the ecological indices entered as independent variables. We used the package REGRESSION (v.2.M) for MATLAB program to implement phylogenetic generalized least-squares (PGLS) analyses as well as ordinary least-squares (OLS) regressions, which are equivalent to assuming that the phylogeny is a star (single hard polytomy) with contemporaneous tips (see Lavin *et al.*, 2008 and Grizante *et al.*, 2010 for details). The package PDAP-PDDIST (Garland & Ives, 2000) in MESQUITE (v2.73; Maddison & Maddison, 2010) was used to generate the phylogenetic variance-covariance matrix used in these analyses. The fit of the two models (PGLS and OLS) was compared based on likelihood-ratio tests (e.g. Grizante *et al.*, 2010), where the model with a higher likelihood indicates a better fit to the tip data. As a rule of thumb, when two regression models have the same number of parameters, one is considered 'significantly' better than other if twice the difference in \ln Likelihoods exceeds 3.841 (the critical value for a χ^2 distribution with 1 d.f. and $\alpha = 0.05$; see Felsenstein, 2004 for details).

The tests detailed previously were carried out using two different topologies, one composed of the 32 species for which diet data were available and another one including all 48 species measured (Fig. 2). Both topologies were based on a topology assembled from Pellegrino *et al.* (2001; for overall relationships among gymnophthalmids) and Kohlsdorf *et al.* (2010; for relationships among *Bachia*), but the following species were replaced due to the lack of their availability in the MZUSP collection: *Gymnophthalmus leucomystax* was replaced by *G. underwoodi* (Kizirian & Cole, 1999; Benozzati & Rodrigues, 2003), *Leposoma guianensis* was used as a sister group of *L. percarinatum* (instead of *L. oswaldoi*, Pellegrino *et al.*, 1999), and *L. scincoides* was included as a external group to dichotomy *L. guianense* + *L. percarinatum* (Pellegrino *et al.*, 2003). Phylogenetic branch lengths in units of divergence times are unavailable in one of the studies used (Pellegrino *et al.*, 2001), and for some of the species replaced (listed above), molecular sequences are not available; therefore, it was impossible to generate a topology with branch lengths proportional to genetic divergence for our study. For these reasons, we tested four different types of arbitrary branch lengths, including all = 1 (constant), Grafen (1989), Pagel (1992) and Nee

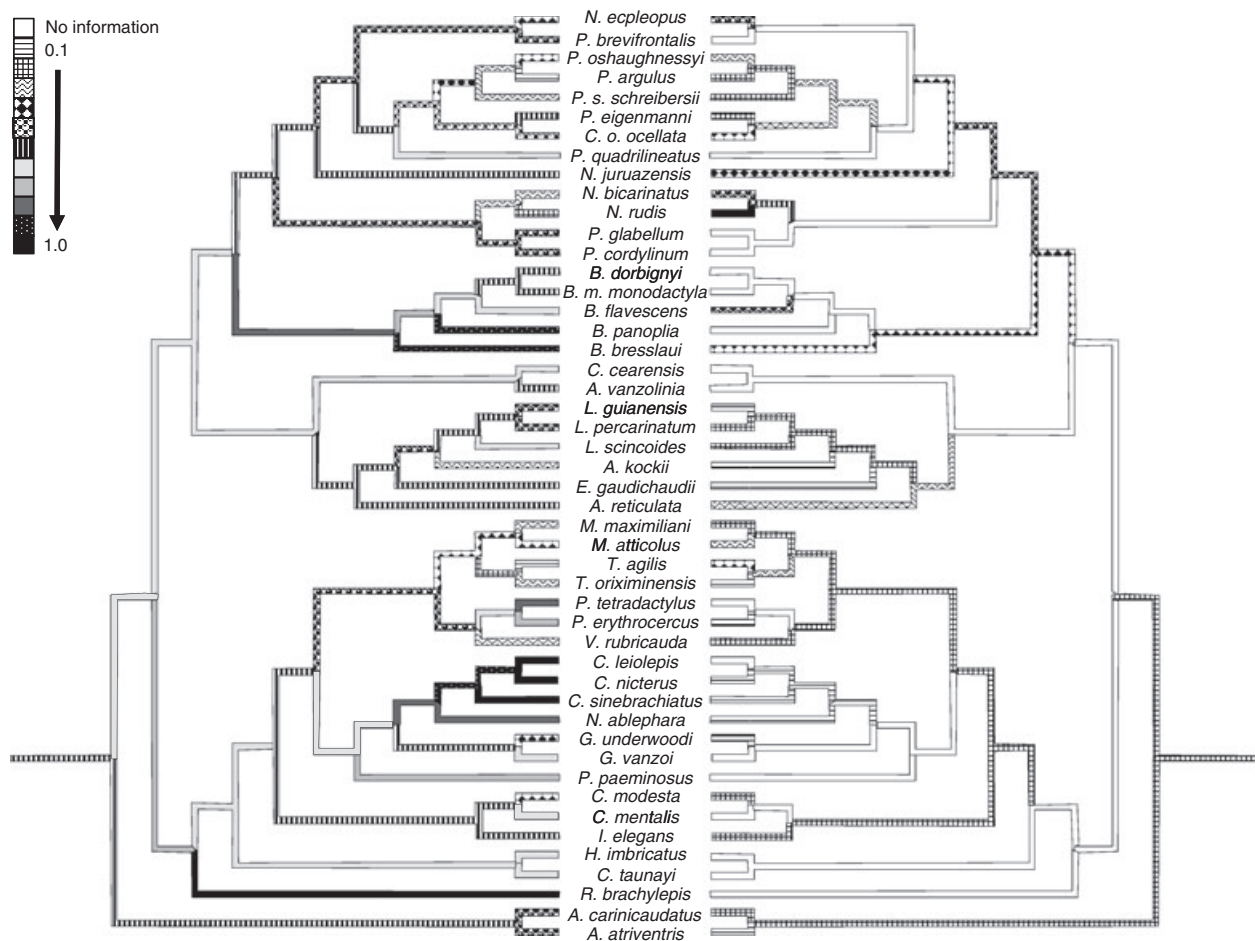


Fig. 2 Topologies used in the phylogenetic analyses with 48 and 32 species. Branch lengths are not assigned. Branch patterning corresponds to ancestral reconstructions based on parsimony (performed in Mesquite v2.73; Maddison & Maddison, 2010); left: microhabitat indexes, right: diet indexes. White branches indicate lineages for which diet information was not available.

(cited in Purvis, 1995), and used diagnostic graphs to verify statistical adequacy of arbitrary branch lengths (Garland *et al.*, 1992); those that better standardized the independent contrasts (Pagel) were used in the analyses.

In this study, multiple comparisons were made using the same data set, and therefore, we estimated the proportion of true null hypotheses using a false discovery rate (FDR) test. The FDR test was implemented using the *QVALUE* software package (Storey, 2002) for R (version 2.9.1; R Development Core Team, 2009), with 'bootstrap' option and the total number of hypotheses under test in the statistical analyses being eight. Significant *q*-values (corresponding to a positive FDR of 5%; Storey, 2002) indicate which significant regressions remain 'true' after correcting the analyses by the number of hypotheses being tested.

The adequacies of the two ecological modes [i.e. diet or microhabitat as a predictor of head shape (PC1 and PC2)] were also directly compared by Akaike Information

Criterion differences (D_i). This test determines the likelihood that a given model is the best model among the candidate models. In order to make the two models comparable, we ran all regressions using the 32 species data set (i.e. excluding all species for which we had data on microhabitat but not diet), then computed the AIC values and calculated ΔAIC (D_i). As a reference, the best model has a D_i value of zero. Models with D_i values up to 2 have substantial empirical support, models with D_i values between 4 and 7 have considerably less support, and models with D_i values > 10 have essentially no empirical support (Burnham & Anderson, 2002). In addition, we used Akaike weights (w_i) to provide another measure of the strength of evidence for each model. Akaike weights (w_i) indicate the probability that a given model is the best among the whole set of candidate models (Burnham & Anderson, 2002). Thus, the best model among all candidates is the one that has the lowest D_i value and the highest w_i .

Table 2 Phylogenetic signal of morphometric traits, using 32 and 48 species and Pagel branch lengths; significant *P* values are indicated in boldface.

	Phylogenetic signal – 32 species				Phylogenetic signal – 48 species			
	<i>P</i>	<i>K</i>	MSE ₀	MSE _{tree}	<i>P</i>	<i>K</i>	MSE ₀	MSE _{tree}
Trunk length	< 0.001	1.414	0.001	0.001	< 0.001	0.743	0.002	0.013
Head length	< 0.001	1.358	0.011	0.004	< 0.001	1.234	0.013	0.005
Snout length	< 0.001	0.935	0.010	0.006	< 0.001	1.012	0.014	0.006
Open in-lever	< 0.001	0.696	0.015	0.011	< 0.001	0.844	0.017	0.009
Close in-lever	< 0.001	1.289	0.032	0.013	< 0.001	1.108	0.029	0.011
Rostral lateral decline	0.251	0.369	0.002	0.002	0.003	0.415	0.002	0.002
Rostral angulation	< 0.001	1.106	0.009	0.004	< 0.001	0.780	0.007	0.004

Table 3 Results of factor analyses with varimax rotation performed on morphometric traits, using data sets with 32 and 48 species; variables with the higher loads in each factor are indicated in boldface.

	32 species – diet		48 species – microhabitat	
	Factor 1	Factor 2	Factor 1	Factor 2
Eigenvalue/ variation (%)	4.254/60.77	1.511/21.58	4.024/57.48	1.313/18.76
Trunk length	-0.108	0.831	-0.123	0.471
Rostral lateral decline	0.314	0.813	0.287	0.828
Rostral angulation	0.758	0.369	0.616	0.580
Head length	0.983	0.063	0.980	0.117
Snout length	0.956	0.136	0.938	0.234
Open in-lever	0.889	0.034	0.900	-0.005
Close in-lever	0.949	-0.014	0.947	-0.031

Results

The evolution of head shape in Gymnophthalmidae seems strongly associated with the topological structure hypothesized for the family, as significant phylogenetic signal was detected for most morphological traits (Table 2), except the rostral lateral decline (analysed for the 32 species tree). These results suggest a trend towards increased resemblance in head shape among phylogenetic closely related Gymnophthalmidae species, as expected.

The factor analyses performed on the morphometric variables retained two components, which were the same when using a data set with 32 or 48 species (Table 3). The first component was positively associated with residuals of jaw closing in-lever, jaw opening in-lever, snout length, head length and rostral angulation. The second factor was positively associated with trunk length and rostral lateral decline (Table 3).

When scores of these components were regressed on ecological indexes (diet and habitat), results from conventional analyses suggest that head shape may be associated with both the microhabitat used and the prey type consumed by Gymnophthalmidae. However, only the significant association between cranial morphology

Table 4 Results of regressions of the scores of the two principal components obtained from factor analyses (Table 3) on the two ecological indexes, using both conventional and phylogenetic (Pagel branch lengths) approaches; significant values are indicated in boldface. Specific *q*-values from the false discovery rate (FDR) test are presented for each *P* value; values lower than 0.05 indicate that the regressions originally assumed as significant remain true when the number of hypotheses tested is considered.

	Factor 1	Factor 2
Diet (32 species)		
Conventional		
<i>t</i> value/ <i>P</i>	3.622/ 0.001	2.062/ 0.048
<i>q</i> -value	0.002	0.040
lnLikelihood	-39.093	-42.778
Coefficient of regression	4.399	2.810
Pagel		
<i>t</i> value/ <i>P</i>	1.806/0.081	0.459/0.649
<i>q</i> -value	0.054	0.309
lnLikelihood	-30.555	-42.116
Coefficient of regression	1.900	0.694
Microhabitat (48 species)		
Conventional		
<i>t</i> value/ <i>P</i>	5.147/ < 0.001	0.003/0.998
<i>q</i> -value	< 0.001	0.416
lnLikelihood	-56.686	-67.604
Coefficient of regression	-2.626	0.002
Pagel		
<i>t</i> value/ <i>P</i>	2.645/ 0.011	0.741/0.463
<i>q</i> -value	0.012	0.257
lnLikelihood	-39.669	-63.222
Coefficient of regression	-1.080	-0.494

and microhabitat usage was retained in our phylogenetically informed analyses (Table 4). Specifically, lizards that occupy microhabitats with greater resistance (e.g. fossorial species) have shorter heads with lower rostral angulation (Fig. 3), similar to the 'round-headed' shapes identified in some amphisbaenids (Kearney & Stuart, 2004). In contrast, the evolution of rostral lateral decline and trunk length (PC2) does not correlate with the ecological indexes used here. Results from the false discovery rate test (*q*-values) corroborated all significant associations identified in the regression analyses (Table 4). As detailed previously, likelihood values can

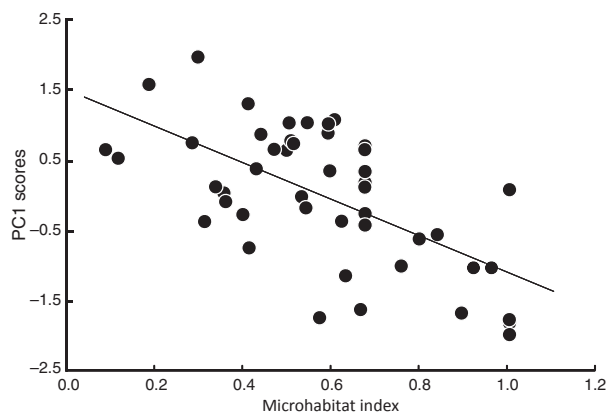


Fig. 3 Correlation between scores of PC1 retained from the factor analysis performed with the 48 species dataset and the index of microhabitat usage.

be used to determine which regression model (conventional or phylogenetic) better explains the evolutionary associations tested, and in our analyses, the lnLikelihood values were always significantly higher in the phylogenetic models (twice the differences always exceeded the critical value of 3.841; see Table 4).

The direct comparison between the two ecological models (diet vs. microhabitat), based on the Akaike Information Criterion differences, strongly supported microhabitat usage as a better predictor of head shape in Gymnophthalmidae, as summarized in Table 5. For PC1, the phylogenetic model with habitat as the predictor had the lowest AIC value (64.24) and a $\Delta AIC (D_i)$ of 0. The phylogenetic model with diet as predictor of head shape had an AIC value of 67 and a $\Delta AIC (D_i)$ of 2.87. Nonphylogenetic models had AIC values over 10 and have no empirical support. The Akaike weights (w_i) also showed that the phylogenetic model with habitat as a predictor is the best model to explain head shape evolution in Gymnophthalmidae (see Table 5). For PC2, both the habitat and diet models had high AIC (diet: 90.23; habitat: 90.27) and low ΔAIC values (diet: 0; habitat 0.02). Akaike weights were similar (diet

Table 5 Summary of results from direct comparisons between the two ecological models, based on Akaike Information Criterion differences (D_i). Analyses performed using the 32 species data set.

Model	AIC	$\Delta AIC (D_i)$	w_i	Interpretation (%)
Factor 1				
Diet (conventional)	84.19	19.95	0.00	0
Diet (phylogenetic)	67.11	2.87	0.19	19
Habitat (conventional)	75.91	11.67	0.00	24
Habitat (phylogenetic)	64.24	0.00	0.81	81
Factor 2				
Diet (conventional)	91.56	1.32	0.19	19
Diet (phylogenetic)	90.23	0.00	0.36	36
Habitat (conventional)	92.72	2.49	0.10	10
Habitat (phylogenetic)	90.27	0.04	0.35	35

$w_i = 0.36$; habitat = 0.35), suggesting that both models have roughly equal probability of being the best model, with the support for the diet model being slightly higher.

Discussion

The present study investigated the effect of potentially conflicting selective pressures of diet and burrowing on the evolution of head shape in gymnophthalmid lizards. Interestingly, several head parameters clustered together in our factor analyses, suggesting that the different features of head morphology are strongly correlated. A robust morphological integration might have functional and/or developmental bases and likely results in a scenario where selection acting on a given parameter of head morphology may result in changes in other traits that define cranial shape. Head shape in this family appears to have evolved in strong association with cladogenetic processes, as significant phylogenetic signal was detected for most morphometric traits (see Garland *et al.*, 2005 for details). Despite the detection of significant phylogenetic signal in the traits studied, association between some parameters of head shape and microhabitat usage was also significant when taking into account the relationships among species. In contrast, associations between head shape and diet were only supported by conventional analyses. Therefore, the evolution of head morphology in Gymnophthalmidae was shaped under the influence of microhabitat use rather than diet (burrowers have shorter heads with lower rostral angulation, independently of the prey consumed). This is in contrast to the clear effect of diet on the evolution of head shape identified in several other lizard groups (e.g. Schwenk, 2000; Herrel *et al.*, 2007; Kohlsdorf *et al.*, 2008).

In Gymnophthalmidae, head shape is not associated with prey hardness, despite the expected changes in the mechanics of the lever system that would be predicted for lizards eating prey with different crushing resistances (Herrel *et al.*, 2001b,c, 2007; Vanhooydonck *et al.*, 2007). In these lizards, jaw closing in-lever (closely tied to bite force) and jaw opening in-lever (associated with the speed of jaw opening) were grouped along the same factor, which was not correlated with diet. Most species of gymnophthalmids, including epigeal lineages as the *Prionodactylus*, appear to eat small and soft prey that move rather slowly (e.g., termites, spiders, larvae, pupae and insect eggs; see Supporting information for details), items that are easy to capture and reduce from a biomechanical perspective (Herrel *et al.*, 1999, 2001b). The selection of softer prey by gymnophthalmids may have relaxed the selective pressures on head shape related to diet and favoured the evolution of fossorial forms in the group, in particular when we assume that the ancestor was not fossorial (see Vidal & Hedges, 2005, for relationships between Gymnophthalmidae and its sister lineage, Teiidae).

In contrast to diet, head morphology is clearly associated with microhabitat use in Gymnophthalmidae, and

the species that use microhabitats with greater resistance to displacement (i.e. fossorial or semi-fossorial lizards that move on sand or under roots and leaf-litter) have shorter heads with lower rostral angulation, which is similar to the 'round-headed' shape identified in amphibaenas (Kearney & Stuart, 2004). In other lineages specialized for life in the underground, head compression and reductions and fusions of cephalic shields are observed (e.g. Gans, 1975; Schwenk, 2000; see also De Schepper *et al.*, 2005, 2007; Herrel *et al.*, 2011 for studies with burrowing fishes), in addition to other morphological changes including trunk elongation (e.g. Gans, 1975; Wiens *et al.*, 2006; Brandley *et al.*, 2008) and limb reduction (e.g. Kearney & Stuart, 2004; Kohlsdorf & Wagner, 2006; Kohlsdorf *et al.*, 2010). Some changes in head morphology (e.g. wedge-shaped snouts) had been previously claimed as fossorial adaptations to sandy environments in the gymnophthalmid lineages *Calyptommatius* and *Bachia* (Rodrigues, 1991; Rodrigues *et al.*, 2007, 2008; Roscito & Rodrigues, 2010), but to our knowledge, this is the first time that effects of likely different selective pressures are considered in a comprehensive investigation of head shape evolution in Gymnophthalmidae.

Microhabitat use was treated in the present study as an index proportional to the percentage of individuals from a given species observed in different substrate categories and the resistance to burrowing implied by each substrate. However, the selective pressures acting during the evolution of head shape in Gymnophthalmidae may be diverse even within the same ecological category. For example, among fossorial lineages, there are burrowers both from dry and sandy Brazilian habitats (e.g. *Calyptommatius* and *Nothobachia* species, all from Caatingas; Rodrigues, 1984, 1991; *Bachia psamophila* and *B. oxyrhina* from Cerrados; Rodrigues *et al.*, 2007, 2008) and from the humid Amazon Forest (e.g. many other *Bachia* species; Kohlsdorf & Wagner, 2006). The biomechanical relationships involved in burrowing activity in these environments are probably quite different, as the soil from rain forests is more compact than the one from loose sand dunes and therefore impinges greater resistance for displacement by the animal's head. The association between burrowing performance, head shape and the microenvironment where the animal is digging has been previously reported in other taxa than squamates: for example, burrowing ability in different lineages of caecilians is directly affected by the soil characteristics (Ducey *et al.*, 1993; Herrel & Measey, 2010), and specifically in the species *Schistometopum thomense*, the males with larger and blunter heads tend to burrow slower (Teodecki *et al.*, 1998). In this context, a promising field of investigation would be testing how general might be the selective pressures acting on the evolution of burrowing performance (and morphological features that underlie this specific mode of locomotion) in squamates when subtle variation in physical properties among substrates is considered.

The discussion presented here emphasizes that selective pressures that act on the evolution of a complex structure such as the cranial system of vertebrates can be different, and even conflicting, when ecological parameters related to diet and microhabitat are considered. The state of knowledge available at this moment provides evidence that the selection of bite force generation capacity plays a major role in shaping cranial morphology in most vertebrate groups (e.g. turtles, Herrel *et al.*, 2002a; fishes, Herrel *et al.*, 2002b; lizards, Kohlsdorf *et al.*, 2008 and Herrel *et al.*, 2007; and birds, Herrel *et al.*, 2009). Among the several possible evolutionary scenarios where the influence of bite force capacity on cranial shape is less pronounced than other selective pressures, one of particular interest relates to animals that burrow in solid substrates using their heads. In the present study, we show that the use of different microhabitats appears to be a determining factor in the evolution of head shape in gymnophthalmid lizards. Interestingly, and in contrast with the evolutionary plasticity in microhabitat usage observed in the family, food preferences appear to be relatively conserved throughout the phylogeny of Gymnophthalmidae (Fig. 2). This pattern has been previously reported for other South American lizards (Vitt *et al.*, 1999), and specifically for microteiids, it may be related to miniaturization in the group. The conservation of food preferences in Gymnophthalmidae may have permitted the extensive radiation of the family into fossorial microhabitats.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Proportions of each microhabitat used (β) and microhabitat index calculated for 48 species of Gymnophthalmidae lizards.

Table S2 Mean and standard error of the resistance forces (in Newtons) measured for each substrate type using a lead model (A_{Fresist}), and the respective prorated resistance force (F_R).

Table S3 Food items eaten by lizards in general, with respective masses and forces for crushing (F_C).

Table S4 Types of prey eaten by gymnophthalmid lizards and diet indexes (DI) calculated.

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