

# Morphological correlates of incipient arboreality and ornithophagy in island pitvipers, and the phylogenetic position of *Bothrops insularis*

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## Abstract

Morphological differences between two semi-arboreal, bird-eating island pitvipers, *Bothrops insularis* and *Gloydus shedaoensis*, and their mainland relatives were investigated. Mitochondrial DNA sequence data for two genes show *B. insularis* to be rooted within *B. jararaca*. *Bothrops insularis* has a more anterior heart, a relatively longer tail, a longer head, and shorter fangs than *B. jararaca*. The greater head length is paralleled in the ecologically similar *G. shedaoensis*. Increased head size may represent an adaptation to the abundance of larger food items (migratory passerine birds), providing a selective advantage to snakes able to switch to larger prey at an earlier age. Furthermore, *B. insularis* and *G. shedaoensis* have converged on similar body sizes from opposite ancestral states. Other characters, including fang length, tail length and size of neonates do not show parallel variation in *G. shedaoensis* and *B. insularis*, suggesting that caution is required when interpreting character state shifts coinciding with ecological shifts in a single species only.

**Key words:** snakes, arboreality, morphology, island species, parallel evolution, *Bothrops*

## INTRODUCTION

Since the beginning of evolutionary biology, islands have played an important part in the formulation and testing of evolutionary theories. For the study of natural selection, one of the most important benefits of islands is that they may provide replicate experiments, where species are subjected to similar selection pressures independently on multiple islands. Where similar patterns of selection result in parallel evolutionary trends, this constitutes strong evidence for the role of natural selection (e.g. Brown, Thorpe & Báez, 1991; Losos *et al.*, 1998).

A benefit of recently isolated islands is that the species found on them are likely to be closely related to, or directly descended from, mainland relatives. Where island forms are recently derived from a morphologically conserved mainland species or species complex, the primitive condition can logically be inferred to lie within the spectrum of variation found among the mainland relatives. Although comparative method analyses can infer primitive conditions and evolutionary trends at higher taxonomic levels (e.g. Martins, Araujo *et al.*, 2001), studies within species or species complexes may reduce errors resulting from homoplasy, and thus better constrain the estimate of the primitive condition.

Among pitvipers, two island species stand out in sharing trophic ecologies and natural history traits differing sharply from those of their mainland relatives. *Bothrops insularis* and *Gloydus shedaoensis* are restricted to the small islands of Queimada Grande and Shedao, off the coasts of south-eastern Brazil and north-eastern China, respectively. Both display pronounced arboreal tendencies (Amaral, 1921; Duarte, Puerto & Franco, 1995; Li, 1995; Martins, Araujo *et al.*, 2001; Martins, Marques & Sazima, 2002; Shine, Sun, Kearney *et al.*, 2002; Shine, Sun, Zhao *et al.*, 2002), and feed almost exclusively on passerine birds as adults, owing to the lack (Queimada Grande) or paucity (Shedao) of mammals on the islands.

Phylogenetically, *Gloydus shedaoensis* is rooted among other species of the morphologically conservative genus *Gloydus*, as the sister species of *G. halys* (Parkinson, Campbell & Chippindale, 2002) or *G. intermedius* (*sensu* Orlov & Barabanov, 1999) (Zhou *et al.*, 2001). Consequently, where a character in *G. shedaoensis* lies outside the spectrum of variation found in mainland *Gloydus*, this must be the result of differentiation since the species became isolated on Shedao.

Recent mtDNA phylogenies confirmed the close relationship between *Bothrops insularis* and the mainland species *B. jararaca* (Salomão *et al.*, 1997, 1999). However, only a single *B. jararaca* haplotype was included in these studies. *Bothrops jararaca* is widespread in south-eastern Brazil, and displays considerable geographic variation

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**Table 1.** MtDNA haplotypes and materials. IB, Coleção Herpetologica Alphonse Hoge, Instituto Butantan, São Paulo, Brazil; BBBSP, Butantan–British *Bothrops* Systematics Project

Haplotype <sup>a</sup>	Species	Locality (Brazil)	Sample information	GenBank accession numbers (cyt <i>b</i> /ND4)
<i>B. neuwiedi</i>	<i>B. neuwiedi</i>	Minaçu, Goiás	IB57513	AF292586, AF292624
<i>B. erythromelas</i>	<i>B. erythromelas</i>	Guanambi, Bahia	IB55541	AF292588, AF292626
<i>jararaca</i> SC	<i>B. jararaca</i>	São Bento do Sul, Santa Catarina	IB55592–3	AY122851, AY122858
<i>jararaca</i> PR1	<i>B. jararaca</i>	Piracuará, Paraná	BBBSP 926	AY122857, AY122864
<i>jararaca</i> PR2	<i>B. jararaca</i>	Piracuará, Paraná	BBBSP 927	AF292589, AF292627
<i>jararaca</i> ES	<i>B. jararaca</i>	Afonso Cláudio, Espírito Santo	BBBSP 918	AY122856, AY122863
<i>jararaca</i> Caman	wild-caught <i>B. jararaca</i> × <i>fonsecai</i> hybrid	Camanducaia, Minas Gerais	IB57374	AY122853, AY122860
<i>jararaca</i> S. Seb	<i>B. jararaca</i>	São Sebastião, São Paulo	BBBSP 1100	AY122855, AY122862
<i>jararaca</i> Emb-G	<i>B. jararaca</i>	Embu-Guaçu, São Paulo	IB55965	AY122852, AY122859
<i>jararaca</i> CMA	wild-caught <i>B. jararaca</i> × <i>neuwiedi</i> hybrid	Campina do Monte Alegre, São Paulo	IB56551	AY122854, AY122861
<i>B. insularis</i>	<i>B. insularis</i>	Ilha da Queimada Grande, São Paulo (3 specimens)	Released after sampling	AF292590, AF292628

<sup>a</sup> Codes as in Fig. 1.

(Hoge, Belluomini & Fernandes, 1976–77; Sazima, 1992). Queimada Grande was only finally separated from the Brazilian mainland by the rise in eustatic sea levels after the last Arctic glaciation (Duarte *et al.*, 1995), suggesting that *B. insularis* may be rooted within *B. jararaca*. Similarly, Shedao Island was repeatedly connected to the Asian mainland during the Pleistocene (Zhao, 1980; Li, 1993).

The convergent natural histories of *G. shedaoensis* and *B. insularis* reflect parallel selective pressures likely to differ from those experienced by mainland snakes. First, both *G. shedaoensis* and *B. insularis* prey almost entirely on birds as adults, whereas juveniles feed primarily on centipedes (Martins, Marques *et al.*, 2002; Shine, Sun, Zhao *et al.*, 2002). Owing to the abundance of migratory birds at certain times of year (Amaral, 1921; Li, 1995; Duarte & Garrubo, 2003), bird-eating adult snakes may be less limited by food availability than juveniles that are too small to eat birds. An ability to switch to avian prey earlier during ontogeny would therefore be selectively advantageous (Sun *et al.*, 2002), and lead to selection for increased neonate size, which is indeed the case in *G. shedaoensis* (Sun *et al.*, 2002). Moreover, since snakes are gape-limited predators (Pough & Groves, 1983), and relative head length determines maximum prey size in vipers (Forsman & Lindell, 1993; Forsman & Shine, 1997), the island forms would be predicted to have longer heads, allowing an earlier switch to avian prey during ontogeny.

In vipers, arboreality is likely to result in selective pressures on other morphological characters, including tail length (Lillywhite & Henderson, 1993; Martins, Araujo *et al.*, 2001), body size and heart position. Body size in arboreal vipers may be constrained owing to the limited haemostatic control in these snakes, and the heart may be predicted to lie in a more anterior position to ensure an adequate blood supply to the brain when the snake is tilted into a head-up position (Lillywhite & Smits, 1992; Lillywhite, 1993; Lillywhite & Henderson, 1993). Thus,

*B. insularis* and *G. shedaoensis* should be smaller, have longer tails, and more anterior hearts than their mainland relatives.

Here, the phylogenetic position of *B. insularis* is analysed, and morphological differences between this species and its mainland relatives are investigated. We also compare the ecologically similar *G. shedaoensis* with related mainland species of *Gloydus* to test for common patterns, which may help elucidate the selective pressures acting during the early stages of the evolution of arboreality and ornithophagy in pitvipers.

## MATERIALS AND METHODS

### Molecular methods

Tissue (liver) or blood samples were obtained from 9 specimens of *B. jararaca* and 3 specimens of *B. insularis*. Specimens, sequences, vouchers and GenBank accession numbers are given in Table 1. Total DNA extraction followed standard methods (Sambrook, Frisch & Maniatis, 1989). Parts of 2 mitochondrial genes, cytochrome *b* (cyt *b*) and NADH dehydrogenase subunit 4 (ND4), were amplified using the polymerase chain reaction (PCR). Primers, PCR conditions and sequencing protocols are as in Pook, Wüster & Thorpe (2000). As outgroups, sequences were used of *Bothrops neuwiedi* and *B. erythromelas*, members of the sister clade to the *B. jararaca*–*insularis* complex (Wüster *et al.*, 2002). Sequences were aligned by eye. The phylogenetic signal was assayed using the *g*1 tree skewness statistic (Hillis & Huelsenbeck, 1992) of all possible trees in PAUP\*4b8 (Swofford, 2001).

Maximum parsimony (MP) and maximum likelihood (ML) methods, implemented in PAUP\*, were used to infer the mitochondrial gene tree of these populations. Exhaustive parsimony searches were executed on the unweighted data only, in view of the low levels of sequence

divergence and consequent lack of saturation of transitions or third codon positions. Bootstrap analysis (Felsenstein, 1985) used 10 000 pseudoreplicates and branch-and-bound searching. Bremer branch support values (Bremer, 1994) were calculated by repeating the exhaustive analysis while retaining successively longer trees, until all nodes were collapsed.

The best model of sequence evolution for maximum likelihood (ML) analysis was estimated using Modeltest 3.0 (Posada & Crandall, 1998). The parameters estimated by Modeltest were used in a branch-and-bound search in PAUP\*. The parameters were then re-estimated from the resulting tree and used in a further branch-and-bound search. ML bootstrap analysis involved 500 pseudoreplicates, heuristic search, tree bisection–reconnection (TBR) branch swapping, and a neighbour-joining starting tree.

Wilcoxon signed-ranks test (WSR; Templeton, 1983) was used to test whether our data significantly reject the hypothesis that *B. insularis* is the sister taxon of all populations of *B. jararaca*, by comparing the optimal trees with trees constrained to support the monophyly of *B. jararaca* to the exclusion of *B. insularis*. For ML, the analogous operation was performed using Shimodaira–Hasegawa tests (Shimodaira & Hasegawa, 1999).

The mean p-distances between sister clades within the *B. jararaca-insularis* group were estimated using the program Phyltest (Kumar, 1996), which was also used for relative rate tests to test for different rates of sequence evolution between sister clades.

### Morphological methods

Preserved material of *Bothrops insularis* ( $n = 27$ ) and *B. jararaca* ( $n = 35$ ), and *Gloydus shedaoensis* ( $n = 5$ ), *G. halys* ( $n = 14$ ), *G. intermedius* (sensu Orlov & Barabanov, 1999;  $n = 4$ ) and *G. brevidaudus* ( $n = 3$ ) was used to compare morphological characters between the arboreal pitvipers and their mainland relatives. Specimens examined are listed in the Appendix.

Snout–vent length (SVL) and tail length were measured to the nearest mm with a piece of string. The length of the head (from the tip of the snout to the end of the quadrate), and the length of the fang (from the tip to the distal edge of the basal orifice; Wüster & Thorpe, 1992) were measured to the nearest 0.01 mm with a set of digital callipers. The position of the heart was recorded as the position of the ventral scale (numbered according to Dowling, 1951) opposite which its posterior tip was situated, and converted to percentage ventral scale (%VS) count to correct for variation in the total ventral scale count. Using ventral scale count rather than a metric measurement of the position of the organ removes the effects of allometric growth and reduces measurement error owing to coiling or twisting of preserved specimens (Thorpe, 1975).

All morphological analyses were carried out using BMDP (Dixon, 1991). The position of the heart tip in *B. insularis* and *B. jararaca* was compared by means of 2-way analysis of variance (2-way ANOVA), using sex and species affinities as grouping variables. Specimens of

*B. insularis* with a hemiclitoris (Böhme, 1995) (formerly termed ‘intersexes’; Hoge, Belluomini, Schreiber *et al.*, 1959) were treated as females.

For size-dependent characters, all measurements were log-transformed to achieve a linear regression, and analysed by means of 2-way analysis of covariance (2-way ANCOVA). Specimens were grouped by sex and species affinities. For *Gloydus*, where species limits remain poorly resolved (compare Gloyd & Conant, 1990, with Orlov & Barabanov, 1999), all specimens were grouped as either *G. shedaoensis* or mainland species. In separate analyses for *Bothrops* and *Gloydus*, comparisons were made of tail length and head length regressed against SVL, and fang length regressed against head length. Both female *G. shedaoensis* had a broken tail, so that tail length differences were only tested in males, by means of 1-way ANCOVA. Finally, the fang length regressed against head length of all *Gloydus* were also compared with the corresponding measurements in *B. jararaca* and *B. insularis*.

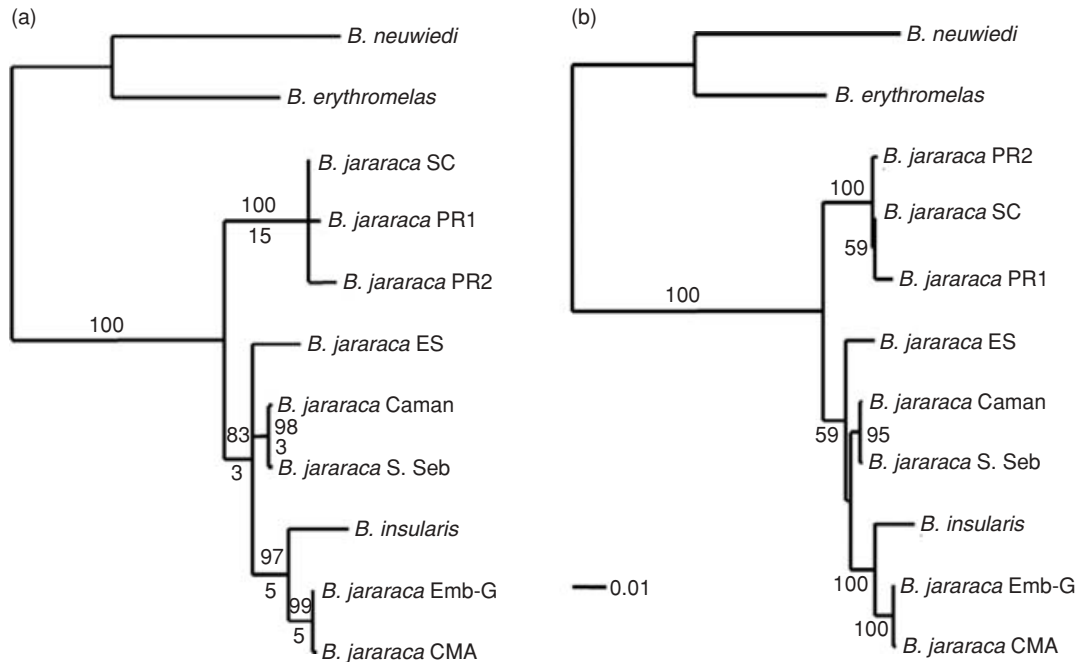
## RESULTS

### Molecular data

For all specimens, 1401 bp (693 of ND4, 708 of *cyt b*) were aligned. Of these, 224 were variable and 117 parsimony-informative. Our data revealed eight unique *B. jararaca* and one *B. insularis* haplotype (Table 1). p-distances between the ingroup and the outgroup taxa ranged from 8.9% to 10.9%. p-distances among ingroup taxa ranged from 0.07% to 3.7%. The distribution of tree lengths was strongly skewed ( $g1 = -2.3125$ , rejecting the null hypothesis of no phylogenetic signal ( $P < 0.01$ ; Hillis & Huelsenbeck, 1992).

The maximum parsimony search resulted in four equally most parsimonious trees (MPTs) of 262 steps (Fig. 1). All trees revealed a basal dichotomy between southern populations of *B. jararaca* (from Santa Catarina and Paraná states) and a northern clade containing the remaining *B. jararaca* and *B. insularis*. In the ML analysis, the GTR +  $\Gamma$  model was identified as optimal for our data. The parameters estimated by Modeltest were used to construct an initial ML tree, and the parameter values recalculated from this tree, and used to estimate the final ML tree ( $-\ln L = 3198.72597$ ). The final ML tree differed from the MP consensus tree in resolving the relationships between the three southern haplotypes, and in placing the Espírito Santo haplotype as the sister group to the other northern *B. jararaca* (Fig. 1).

The two most parsimonious constrained trees retaining the *B. jararaca* haplotypes as a clade to the exclusion of *B. insularis* had a length of 270 steps. Pairwise WSR tests comparing each constrained tree with each MPT revealed mostly significant pairwise comparisons ( $-Z = 1.8856-2.3094$ ;  $P = 0.0209-0.0593$ ), only one out of eight being insignificant. The analogous ML-based Shimodaira–Hasegawa test approached significance ( $d(-\ln L) = 9.28457$ ,  $P = 0.06$ ).



**Fig. 1.** (a) Maximum parsimony and (b) maximum likelihood tree for the *Bothrops jararaca* and *B. insularis* haplotypes found in this study. Localities: Caman, Camanducaia, Minas Gerais; CMA, Campina do Monte Alegre, São Paulo; Emb-G, Embu-Guaçu, São Paulo; ES, Afonso Cláudio, Espírito Santo; PR, Piracuara, Paraná; SC, São Bento do Sul, Santa Catarina; S. Seb, São Sebastião, São Paulo.

**Table 2.** Morphological summary data. For linear measurements, mean values are given adjusted to the mean snout–vent length (or head length for fang length) of all specimens, separately for *Bothrops* and *Gloydius*

	<i>B. insularis</i> males (n = 11)	<i>B. insularis</i> females (n = 16)	<i>B. jararaca</i> males (n = 12)	<i>B. jararaca</i> females (n = 23)	<i>Gloydius</i> <i>shedaoensis</i> males (n = 3)	<i>Gloydius</i> <i>shedaoensis</i> females (n = 2)	Other <i>Gloydius</i> males (n = 11)	Other <i>Gloydius</i> females (n = 10)
Mean adjusted tail length (mm)	124.9	110.2	115.3	102.9	74.44	n/a	74.97	n/a
Mean adjusted head length (mm)	34.4	38.4	32.5	34.0	30.88	27.04	25.80	25.11
Mean adjusted fang length (mm)	6.22	6.47	7.56	8.48	3.81	4.27	4.40	4.19
Mean heart position (%VS)	32.51	29.88	33.19	31.22	33.12 (n = 1)	30.76 (n = 1)	33.15	31.24

## Morphological characters

The results of all morphological analyses are shown in Tables 2–4. The heart is situated in a significantly more anterior position in *B. insularis* than in *B. jararaca*, and more anteriorly in females than in males (Fig. 2). The heart had been removed in all but one male and one female specimen of *G. shedaoensis*, and there was no significant difference between this species and other *Gloydius*. *Bothrops insularis* has a significantly longer tail, a significantly longer head, and significantly shorter fangs than *B. jararaca* (Figs 3–5). *Gloydius shedaoensis* has a significantly longer head than other species of the genus (Fig. 4), but there is no clear pattern for fang or tail length. The fangs of all *Gloydius* are significantly shorter relative to head length than those of *B. jararaca*, but do not differ significantly from those of *B. insularis*.

## DISCUSSION

Our data confirm that *Bothrops insularis* is a relatively recently isolated derivative of *B. jararaca*. The southern *B. jararaca* haplotypes differ by a mean p-distance of 2.79% from the *B. insularis* and northern *B. jararaca* haplotypes. This is consistent with a late Pliocene/early Pleistocene divergence, depending on rates of sequence evolution. A rate of 1.4% My<sup>-1</sup> for cyt *b* and ND4 (Wüster *et al.*, 2002) would suggest a basal divergence *c.* 2 million years ago. However, given the error margins of molecular clocks (Hillis, Mable & Moritz, 1996), this cannot be regarded as anything other than a very rough approximation.

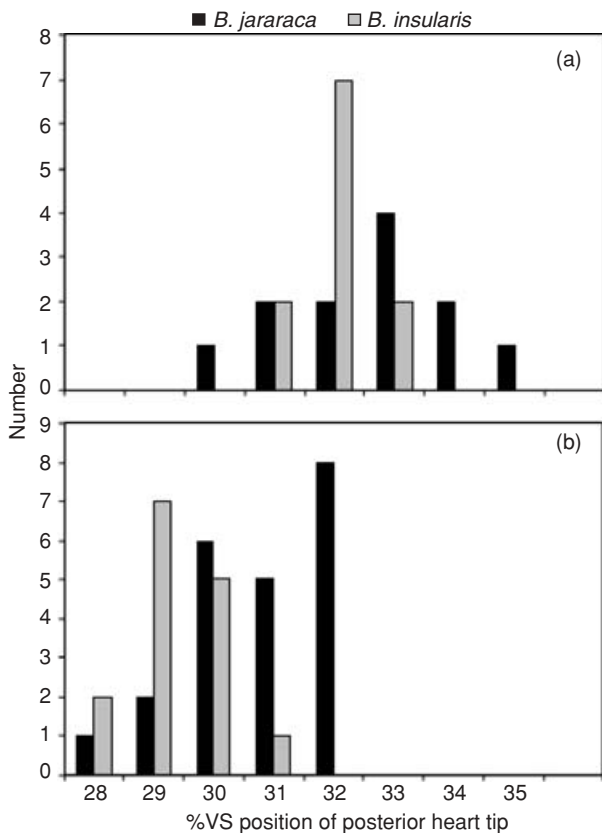
Although our sampling of mainland populations was relatively coarse, *B. jararaca* displays clear phylogeographic structure across its range. This species also shows

**Table 3.** Results of ANOVA and ANCOVA of morphological data, comparing *B. insularis* with *B. jararaca*, and *G. shedaoensis* with other *Gloydus*. Degrees of freedom in each analysis is 1. Bold type indicates significant results

	Species		Sex		Species × sex	
	F	P	F	P	F	P
<i>Bothrops insularis</i> vs <i>B. jararaca</i>						
Heart position	12.45	< <b>0.001</b>	64.67	< <b>0.0001</b>	1.36	0.2488
Head length vs SVL	55.50	< <b>0.0001</b>	40.55	< <b>0.0001</b>	8.59	<b>0.0049</b>
Tail length vs SVL	18.48	< <b>0.0001</b>	48.35	< <b>0.0001</b>	0.13	0.7155
Fang length vs head length	98.62	< <b>0.0001</b>	7.81	<b>0.0071</b>	2.62	0.1112
<i>Gloydus shedaoensis</i> vs other <i>Gloydus</i>						
Heart position	0.08	0.7759	5.76	<b>0.0268</b>	0.06	0.8038
Head length vs SVL	9.15	<b>0.0064</b>	3.90	0.0615	1.64	0.2140
Tail length vs SVL	0.683	0.426	n/a	n/a	n/a	n/a
Fang length vs head length	1.85	0.1891	0.72	0.4052	4.32	0.0508

**Table 4.** Results of ANCOVAs of fang length regressed against head length, comparing all *Gloydus* with *B. insularis* and with *B. jararaca*. Degrees of freedom in each analysis is 1. Bold type indicates significance

	Taxon		Sex		Taxon × sex	
	F	P	F	P	F	P
Fang length vs head length: <i>Gloydus</i> vs <i>B. insularis</i>	0.08	0.7785	0.01	0.9352	0.64	0.4267
Fang length vs head length: <i>Gloydus</i> vs <i>B. jararaca</i>	39.57	< <b>0.0001</b>	2.21	0.1429	5.01	<b>0.0293</b>



**Fig. 2.** Position of the posterior heart tip in relation to ventral scale count in (a) male and (b) female *Bothrops insularis* and *B. jararaca*.

considerable geographic variation in some morphological characters, which has been interpreted as a result of direct environmental induction by current climatic conditions

(Hoge, Belluomini & Fernandes, 1976–77). Such an interpretation is clearly premature: the distribution of low ventral scale counts seems to coincide with the southern haplotype lineage, suggesting that these populations may represent a distinct evolutionary lineage potentially deserving of taxonomic recognition. However, the definition of species limits based solely on mtDNA phylogeographic patterns, without critical analysis of unlinked data, is premature (Puerto *et al.*, 2001). A thorough study of the population systematics of *B. jararaca* is urgently required, especially in view of the medical importance of this species (Fan & Cardoso, 1995; Warrell, 2004).

*Bothrops insularis* differs considerably from *B. jararaca* in various aspects of morphology. Paralleling general trends in the genus *Bothrops* (Martins, Araujo *et al.*, 2001), the arboreal *B. insularis* has a longer tail than the less arboreal *B. jararaca*. The more anterior position of the heart is consistent with general observations on arboreal snakes, in which the more anterior heart position may facilitate cerebral blood supply (Lillywhite, 1987) or heart filling (Badeer, 1998). The significantly greater difference in heart position between the larger females of *B. insularis* and *B. jararaca* (1.34%VS), compared to the smaller males (0.68%VS) is also consistent with this hypothesis. Unfortunately, no comparable data are available for *G. shedaoensis*.

As predicted, both *B. insularis* and *G. shedaoensis* display an increase in head length relative to their mainland relatives. The birds on which adults feed are migratory, and available in large numbers at certain times of the year. They are of no benefit, however, to smaller juvenile snakes, which are too small to eat adult passerine birds. Relative head length has been shown to be a key

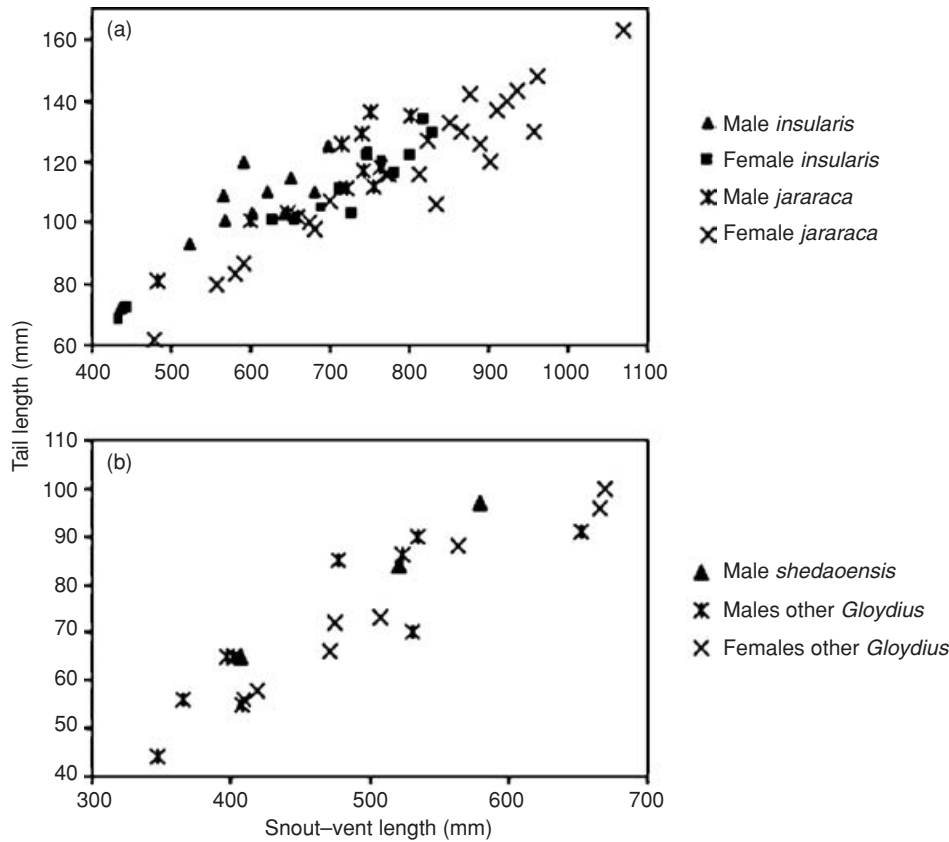


Fig. 3. Tail length plotted against snout–vent length: (a) *Bothrops insularis* and *B. jararaca*; (b) *Gloydus shedaoensis* and other *Gloydius*.

determinant of maximum prey size in snakes (Forsman & Lindell, 1993). The larger head in the island species would thus allow juveniles of these island species to switch to the more abundant avian prey at an earlier age and smaller body size.

The reduced fang length of *B. insularis* compared to *B. jararaca* could also be related to foraging mode. *Bothrops jararaca* uses strike–release–track behaviour on terrestrial mammalian prey (Sazima, 1992), whereas *B. insularis* retains avian prey in its mouth after envenoming, in a manner akin to that of elapid snakes. In that context, shorter fangs may reduce the risk of fang breakage caused by a struggling prey item. Alternatively, the fact that the snake retains the prey in its mouth may reduce the need for rapid fang penetration, and thus for long fangs.

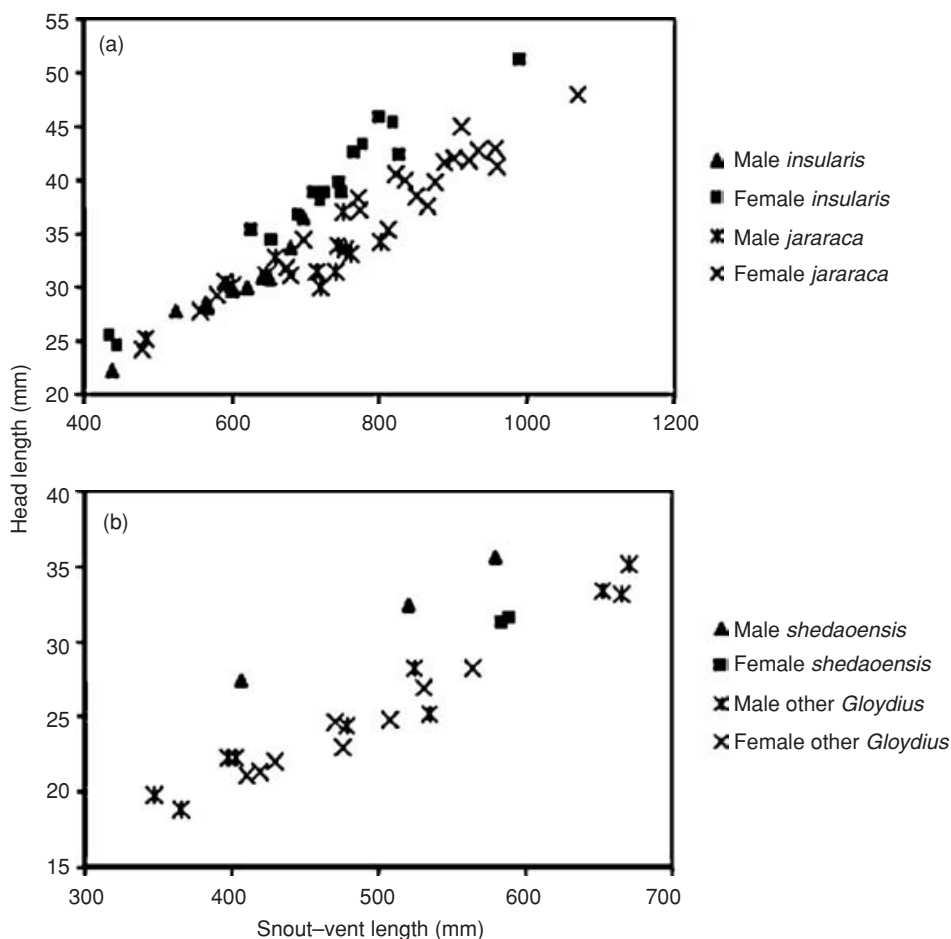
In *G. shedaoensis*, the length of the fang does not differ significantly from that of other species of the genus. Moreover, there is no indication that this is a result of the small sample size rather than the absence of a difference. Interestingly, in all species of *Gloydius*, the relationship between fang length and head length is similar to that of *B. insularis*, but the fangs are shorter in relation to head length than in *B. jararaca*. This raises the possibility of an optimal relationship between fang length and head size for different foraging modes, and in particular for viperids that retain prey in their mouths after the initial strike. A broader study of the relationship between fang length and foraging mode among viperids would be of considerable interest in this context.

### Other characters associated with arboreality and ornithophagy

#### Reproduction

Compared to its mainland relatives, *G. shedaoensis* has fewer and larger young in each litter, which has been interpreted as an adaptation enabling an earlier shift to abundant, larger avian prey (Sun *et al.*, 2002), complemented by the increased head size demonstrated here. In *Bothrops insularis*, there is evidence of reduced litter size (see also Almeida-Santos & Salomão, 2002): Hoge, Belluomini, Schreiber *et al.* (1959) counted 106 embryonated eggs in the ovaries of 18 dissected females (mean 5.89, SD 3.10), and noted one litter of five being born. For *B. jararaca*, the mean litter size has been reported as 13.5 ( $n = 49$ ; Breno *et al.*, 1990), 10.75 ( $n = 8$ ; Sazima, 1992), and 21 ( $n = 10$ ; Cardoso, 2001).

Despite the smaller litter size, however, there is no evidence for increased neonate size in *B. insularis*: Sazima (1992) indicates a mean SVL of 245 and 253 mm and mean weight of 9.38 and 10.61 g for male and females of *B. jararaca*, and Janeiro-Cinquini, Leinz & Ishizuka (1990) give a mean SVL of 240 and 241 mm and mean weights of 7.94 and 8.02 g for males and females, respectively. Published data for *B. insularis* litters are lacking. Two litters of seven and two neonates, respectively, born in captivity at Instituto Butantan, had a mean SVL of 228 mm and a mean weight of 9.11 g (sexes not separated; pers. obs.). Clearly, more information on neonate size in



**Fig. 4.** Head length plotted against snout–vent length: (a) *Bothrops insularis* and *B. jararaca*; (b) *Gloydus shedaoensis* and other *Gloydus*.

*B. insularis* is required, but at present, there is no indication that neonate *B. insularis* are longer or heavier than neonate *B. jararaca*. The lack of parallelism in neonate body size in these two species is surprising, and difficult to explain in terms of selection regimes on the islands. Potential alternative causes include physiological constraints on gravid female *B. insularis*, or the effects of prolonged inbreeding in this species (Hoge, Belluomini, Schreiber *et al.*, 1959).

#### Adult size

Owing to the relatively posterior position of the heart and the poor haemostatic control of viperid snakes, arboreality in this family is normally associated with small size (Lillywhite & Smits, 1992; Lillywhite, 1993; Lillywhite & Henderson, 1993). This would predict that, if anything, *B. insularis* and *G. shedaoensis* should be smaller than their mainland relatives. This prediction is fulfilled in *Bothrops*: *B. jararaca* is considerably larger (maximum total length *c.* 160 cm, Campbell & Lamar, 1989; mean female SVL 947 mm, Martins, Araujo *et al.*, 2001) than *B. insularis*: in a sample of 368 live, field-caught specimens of the latter the average female SVL was 683 mm, and the maximum total length was 1095 mm. A preserved female specimen, IB 46481, had a SVL of 990 mm; the tail was

incomplete, but would have measured *c.* 140 mm intact. Females grow considerably larger than males, as in other species of *Bothrops*.

In contrast, *Gloydus shedaoensis* grows larger than its mainland relatives, with a mean SVL of 650–700 mm (Shine, Sun, Kearney *et al.*, 2002; Shine, Sun, Zhao *et al.*, 2002) and a record total length of 99 cm (Shine, Sun, Zhao *et al.*, 2002), making this the largest species of *Gloydus*.

The reduced size of *B. insularis* relative to *B. jararaca* is as predicted from studies of the haemodynamics of viperid snakes (Lillywhite & Smits, 1992), but the increased size of *G. shedaoensis* is not. It is notable, however, that the size of *G. shedaoensis* is similar to that of *B. insularis*. This convergence may be the result of a trade-off between physiological constraints and the need to be able to consume relatively large avian prey. The reduced size of *B. insularis* may thus be the result of selection for a size more compatible with increased arboreality, whereas *G. shedaoensis*, originating from mainland stock smaller than any physiologically critical length for arboreal viperids, may have evolved an increased body size as a result of selection for the ability to exploit avian prey.

Clearly, other causes, such as trophic richness or selection for different levels of reproductive effort (Sun *et al.*, 2002), may also have influenced the maximum size of these island snakes. Nevertheless, it is striking that these

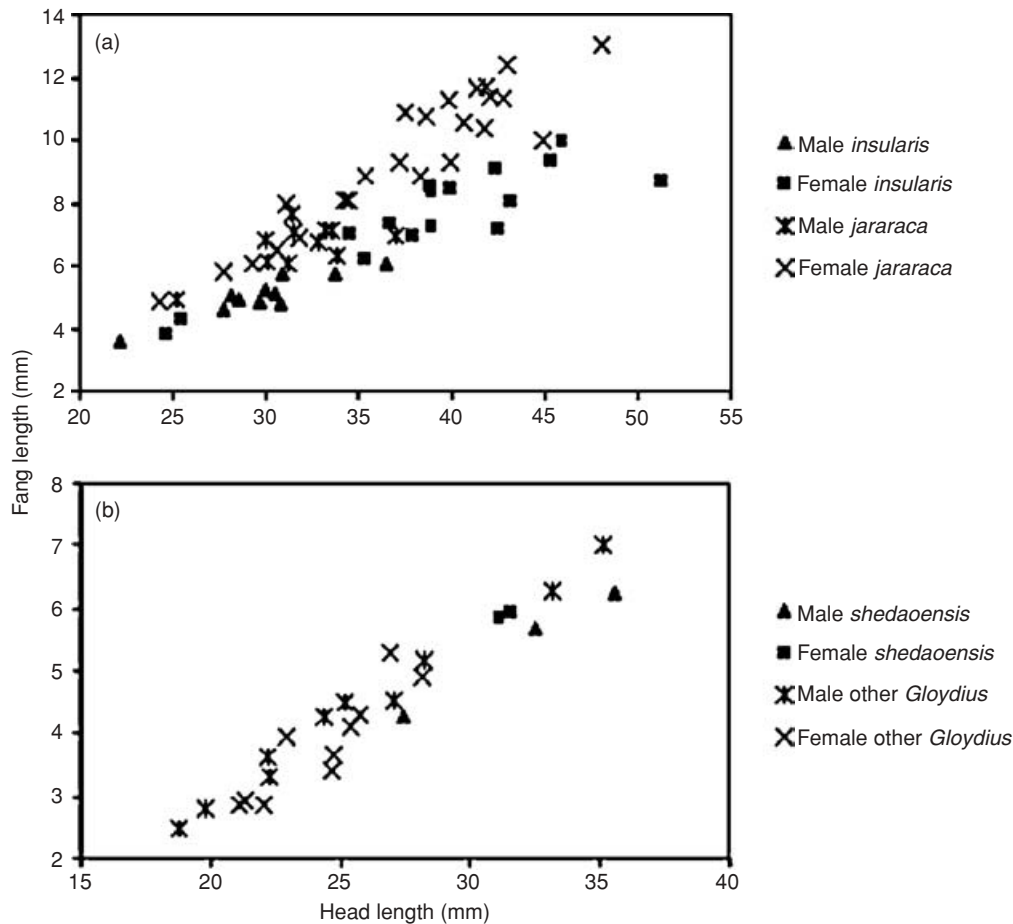


Fig. 5. Fang length plotted against head length: (a) *Bothrops insularis* and *B. jararaca*; (b) *Gloydius shedaoensis* and other *Gloydius*.

two semi-arboreal species reach similar lengths, despite phylogenetic distance and profound differences in habitat, climate, and the size and stature of mainland relatives.

## CONCLUSIONS

Comparative studies, which analyse correlations between differing natural histories and morphological characters in phylogenetically well-characterized groups, have the potential to highlight the role of natural selection in shaping the evolution of morphological characters. In this study, we have demonstrated parallel or convergent changes in some aspects of the morphology and life history of two recently isolated island pitvipers with similar arboreal, ornithophagous habits. These comparisons have helped to shed further light on the selective pressures acting on these snakes, and on their effects in the evolution of morphological novelties, but examples of lack of parallel evolution urge caution in the interpretation of results from the studies of single species.

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- APPENDIX.** Specimens examined for morphological comparisons
- Bothrops insularis* (all collection numbers from Instituto Butantan): BRAZIL: **São Paulo**: Ilha da Queimada Grande: 17168–9, 17781, 17883, 18184, 18394, 18481, 18491, 18495, 18497, 18551, 18646, 19021, 19510, 20778, 42900, 44268, 44366, 44392, 44399, 44401, 45303, 45640, 45980, 46134, 46481, 54964.
- Bothrops jararaca* (all collection numbers from Instituto Butantan): BRAZIL: **Espírito Santo**: Domingos Martins: Marechal Floriano: 28349–52, 28354–6, 28359–60; Fundão: 16060; São Domingos: Águia Branca: 24213; **Paraná**: Arapoti: 55395–400, 55402–3; **Santa Catarina**: Chiqueirão: 13516; Fragosos: 13411; Herval d'Oeste: 56634–6; Mafra: 13401; Nova Galícia: 13357; Rio Azul: 13407; Santa Cruz do Timbo: 13311; São Bento do Sul: 444; **São Paulo**: Bananal: 55505; Belém: 738; Miracatu: 56835; Miracatu: Jaracotia: 28965, 28969; São João: 13252.
- Gloydus shedaoensis*: CHINA: **Liaoning**: Shedao Island (ROM 20468–20472).
- Gloydus halys*: AFGHANISTAN: **Herat**: Sausak Pass: ZMUC r 6912. CHINA: **Shansi**: 12 miles NW Kholan Chow: BM 1908.11.28.1.1; **Sinkiang Autonomous Region**: Tien Shan Mountains: BM 1904.11.1.34, 1904.11.1.36–38; RUSSIA: Altai: NRM 1806a-d; Smeinogorsk: ZMUC R 6949; TURKMENISTAN: Kopet Dag: Ai Dara: BM 92.11.28.12; Mangyschak Peninsula: BM 1920.1.20.257, ZMUC R 695.
- Gloydus intermedius*: KOREA: Engan: NRM SBM 1935.222.3031; RUSSIA: Khabarovsk: BM 89.12.16.140–141; Ussuri River: BM 75.10.14.1;
- Gloydus brevicaudus*: CHINA: **Anhui**: Chu Chow: NRM 2595; Tung-Ling Hsien: Chung Ming Chich: NRM 2591a-b.