Herpetologica, 68(3), 2012, 418–429

A NEW AND THREATENED INSULAR SPECIES OF LANCEHEAD FROM SOUTHEASTERN BRAZIL

Fausto E. Barbo[1,2,7], Felipe G. Grazziotin[2,3], Ivan Sazima[4], Marcio Martins[5], and
Ricardo J. Sawaya[6]

[1]*Programa de Pós-graduação em Biologia Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), 15054-000 São José do Rio Preto, SP, Brazil*
[2]*Museu de Zoologia, Universidade de São Paulo (MZUSP), P.B. 42494, 04218–970 São Paulo, SP, Brazil*
[3]*Programa de Pós-graduação em Zoologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), 13506–970 Rio Claro, SP, Brazil*
[4]*Museu de Zoologia, Universidade Estadual de Campinas, 13083–970 Campinas, SP, Brazil (retired and associated as researcher)*
[5]*Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, 05508–090 São Paulo, SP, Brazil*
[6]*Departamento de Ciências Biológicas, Universidade Federal de São Paulo (UNIFESP), 09972–270 Diadema, SP, Brazil*

Abstract: We describe a new species of *Bothrops* from Vitória Island, off the coast of São Paulo, southeastern Brazil. The new species differs from the mainland coastal populations of *B. jararaca* mostly in its smaller and stouter body, number and form of scales, and hemipenial morphology. From *B. insularis* and *B. alcatraz*, both related species endemic to islands in southeastern Brazil, *B. otavioi* sp. nov. differs mainly in its body form and number of scales. The new species has the most common mitochondrial haplotype for mainland populations of *B. jararaca*, which is also found in *B. alcatraz*. A mitochondrial genealogy (gene tree) shows the new species nested within the northern clade of *B. jararaca*. This genealogical pattern can be explained by a recent speciation event for *B. otavioi* sp. nov. The isolation of insular species of *Bothrops* from continental ancestor populations are probably related to the same vicariant process, the oscillations of sea level during the Pleistocene. The new species feeds on small hylid frogs, and attains sexual maturity at 388 mm snout–vent length (SVL; males) and 692 mm SVL (females). *Bothrops otavioi* sp. nov. is endemic to Vitória Island, and should be listed as critically endangered because it is known from only a single area (an island), its geographic range covers less than 100 km^2, and there is a projected continuing decline in the quality of its habitat because of increasing human settlement.

Key words: Atlantic Forest; *Bothrops jararaca* group; *Bothrops otavioi* sp. nov.; Evolution; Island endemics; Quaternary; Taxonomy

The genus *Bothrops* contains about 50 species distributed in Central and South America, of which 6 species groups are formally recognized: *B. alternatus*, *B. atrox*, *B. jararaca*, *B. jararacussu*, *B. neuwiedi*, and *B. taeniatus* groups (Campbell and Lamar, 1989; Salomão et al., 1997, 1999; Campbell and Lamar, 2004). The *B. jararaca* species group has a widespread distribution in Brazil, from the coastal portion of southern Bahia to northern Rio Grande do Sul, reaching inland mainly in São Paulo and Minas Gerais (Marques et al., 2002; Grazziotin et al., 2006). Its distribution is highly congruent with the Atlantic Forest biome of Brazil (Campbell and Lamar, 2004). Species within the *B. jararaca* group also occur on some continental islands (Duarte et al., 1995; Marques et al., 2002; Cicchi et al., 2007).

Some island populations of *B. jararaca* seem to be very similar to mainland populations, although a few insular forms are diagnosable (see Marques et al., 2002; Grazziotin et al., 2006). Because these populations may represent isolated evolutionary units (there is no evidence of gene flow to these islands), they should be recognized as full species. Presently, the *B. jararaca* group comprises the following species: the widespread mainland *B. jararaca* (Salomão et al., 1997; Campbell and Lamar, 2004; Grazziotin et al., 2006), which is probably a complex of species (see Grazziotin et al., 2006), and three island endemics off São Paulo coast: *B. insularis* from Queimada Grande Island, *B. alcatraz* from Alcatrazes Island, and a new species from Vitória Island that we describe herein.

Aiming to allocate the species of a clade composed of the *Bothrops jararaca* + *B. neuwiedi* groups, Fenwick et al. (2009) pro-

---

[7] Correspondence: e-mail, faustoebarbo@gmail.com

posed a new genus, *Bothropoides*, diagnosed by nonunique phenotypic synapomorphies such as lepidosis, hemipenial morphology, cranial osteology, dentition, coloration, and 38 mitochondrial characters. Those authors also resurrected the genus *Rhinocerophis* Garman 1881 to allocate the species of *B. alternatus* group, diagnosed by 27 mitochondrial characters and one–two palatine teeth as unique morphological synapomorphy. Based on new data, Carrasco et al. (2010) pointed out that the morphological analysis by Fenwick et al. (2009) was incomplete and rejected the use of *Rhinocerophis*, which made the *Bothrops* complex paraphyletic with respect to *Bothriopsis* and *Bothropoides* (Carrasco et al., 2010). The lack of an exclusive morphological character in *Rhinocerophis* and *Bothropoides,* and a diagnosis based only on molecular data, indicates that splitting of the genus *Bothrops* into two or more genera is still premature. More recently, Carrasco et al. (2012) examined 111 characters of lepidosis, and 2393 molecular characters from South American bothropoid snakes. Their results pointed out that *Bothrops* sensu stricto is paraphyletic, and proposed new rearrangements to rectify this paraphyly, maintaining *Bothrocophias*, and synonymizing *Bothriopsis*, *Bothropoides*, and *Rhinocerophis* with *Bothrops*. Therefore, we maintain here the generic name *Bothrops* for the *B. jararaca* group (cf. Wüster et al., 2002; Carrasco et al., 2012), instead of adopting the rearrangements within this genus proposed by Fenwick et al. (2009).

Materials and Methods

We examined 30 specimens of the new species housed in the reptile collections of the Instituto Butantan (IBSP), the Museu de Zoologia da Universidade de Campinas (ZUEC), and the Museu de Zoologia da Universidade de São Paulo (MZUSP), and 210 specimens ascribed to *B. jararaca* from adjacent mainland populations of the São Paulo coast (Fig. 1; Appendix).

We measured snout–vent length (SVL), tail length (TL), and total length (TTL=SVL+TL) to the nearest millimeter with a flexible ruler. We measured head length (HL; snout to extreme posterior portion of mandible), and eye diameter (ED) with calipers to the nearest 0.1 mm. We determined the trunk length (TR) as SVL – HL. We performed statistical tests with Statistica 5 and Statistica 6 (Statsoft, 1995, 2001). We tested for differences in SVL between species with a Student's *t*-test. To compare relative lengths of the tails and heads between species, we used the ratios TL/SVL and HL/TR, respectively. We tested for differences in relative lengths of tails and heads between sexes of the new species using analyses of covariance (ANCOVA), in which SVL and TR were the covariates, respectively (Zar, 1996).

Description and scale counts follow Campbell (1985). We counted ventral scales beginning with the first scale that was wider than long. We counted anterior cephalic scales (from posterior edge of supraocular scales to posterior edge of intercanthal scales; Fig. 2), intercanthal scales (from posterior edge of intercanthal scales to snout), and intersupraocular scales (line of scales in the middle, between supraoculars). We also counted the following lateral head scales: interoculabial scales (between top of fourth and fifth supralabial scales and circumorbital scales), circumorbital scales (scales contacting eye), pre- and postfoveal scales, temporal scales, and supra- and infralabial scales. We derived information on prey type and reproduction from preserved specimens.

We extracted from liver tissue with the use of standard methods for DNA extraction (Sambrook and Russell, 2001) based on enzymatic digestion (proteinase K) and deproteinization (phenol:chloroform). We amplified approximately 700 base pairs for the mitochondrial gene cytochrome b (*cytb*) with the use of primers and protocol described by Pook et al. (2000). We purified the PCR product enzymatically and processed the sequence using DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare) in a MegaBACE 1000 automated sequencer (GE Healthcare) following manufacturer's protocols. The quality of both strands was assessed and assembled with the use of the program Geneious 5.4 (www.geneious.com). We included our sequence in the molecular matrix used by Grazziotin et al. (2006) and aligned our sequence with the use of profile alignment as implemented in Clustal X v2 (Larkin et al., 2007). Our analysis of haplotype relationship was based on a probabilistic approach. We

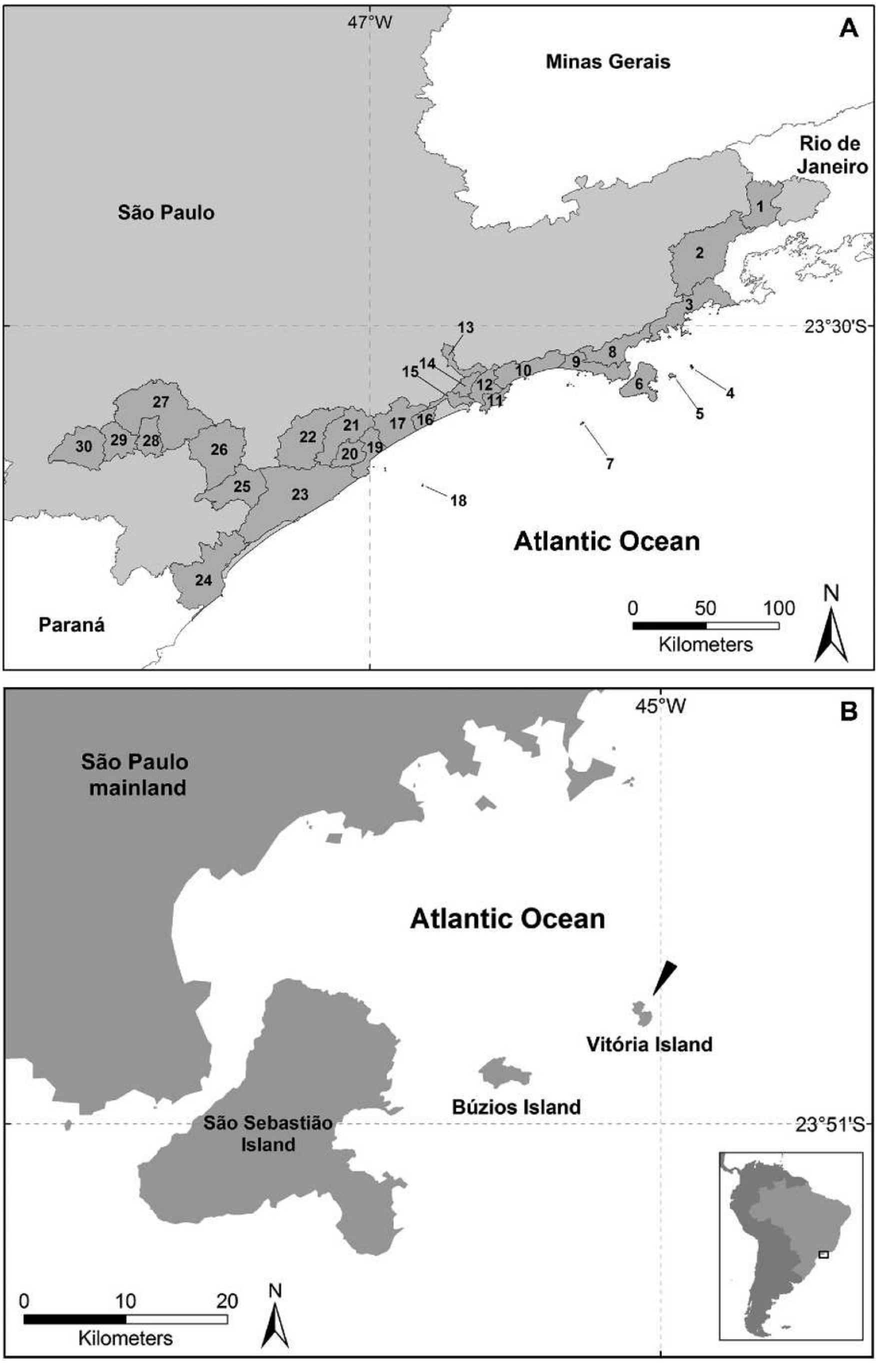

FIG. 1.—Coastal region of São Paulo, showing mainland and island localities of examined specimens. (1) São José do Barreiro; (2) Cunha; (3) Ubatuba; (4) Vitória Island; (5) Búzios Island; (6) São Sebastião Island; (7) Alcatrazes Island; (8) Caraguatatuba; (9) São Sebastião; (10) Bertioga; (11) Guarujá; (12) Santos; (13) Santo André; (14) Cubatão; (15) São Vicente; (16) Mongaguá; (17) Itanhaém; (18) Queimada Grande Island; (19) Peruíbe; (20) Itariri; (21) Pedro de Toledo; (22) Miracatu; (23) Iguape; (24) Cananéia; (25) Registro; (26) Sete Barras; (27) Capão Bonito; (28) Ribeirão Grande; (29) Guapiara; (30) Ribeirão Branco (A); Vitória Island, adjacent islands, and coastal mainland (B).

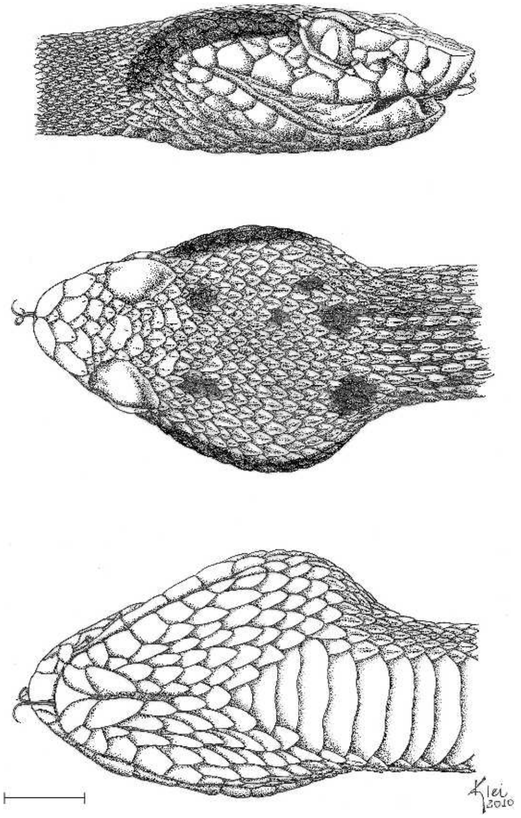

FIG. 2.—Lateral, dorsal and ventral views of the head of the holotype of *Bothrops otavioi* sp. nov. (IBSP 78572). Note the diagnostic (unique) large scale on the dorsal side of head, between the supraoculars. Line = 5 mm.

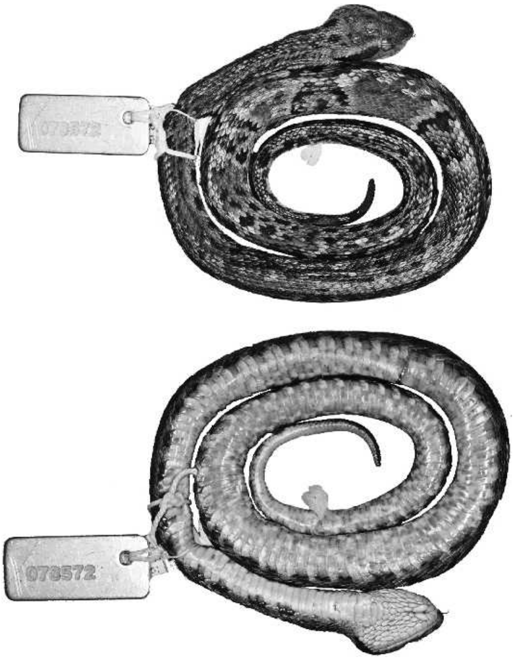

FIG. 3.—*Bothrops otavioi* sp. nov. (holotype, IBSP 78572): Dorsal and ventral views. snout–vent length = 391 mm, tail length = 65 mm. Photographs by Fausto E. Barbo.

used a maximum-likelihood method implemented in PAUP* (Swofford, 2002), as used by Grazziotin et al. (2006).

Results

Bothrops **otavioi** *sp. nov.*
(Figs. 2–4A, Table 1)

Bothrops jararaca, Cicchi et al., 2007:227–240; in part.

Holotype.—IBSP 78572 (field number CC58), male, collected 11 August 2010 by F.C. Centeno and T.H. Condez, from “Trilha da Vitória” (23°44′28″S, 45°01′16″W; datum = WGS 84; Figs. 1 and 4B), Vitória Island, Ilhabela Archipelago, São Paulo, southeastern Brazil (Figs. 2–4A). We deposited a tissue sample at the herpetological collection of MZUSP.

Paratypes.—Twenty specimens from the type locality: IBSP 18866–67, males; IBSP 18868, female; IBSP 18870–71, females; IBSP 18872–73, males; IBSP 18874–75, females; IBSP 18876–77, males; IBSP 18879–81, females; IBSP 18882, male, and ZUEC 3551 female, collected March 1960 by A.R. Hoge (Instituto Butantan expedition); MZUSP 3949 female, MZUSP 3951 male; MZUSP 3952 female, and ZUEC 3550 male, collected 14–28 March 1964 by the Departamento de Zoologia (DZ) expedition.

Referred specimens.—Ten specimens from the type locality: IBSP 18878 (flattened specimen), March 1960, Instituto Butantan expedition collection; MZUSP 5577–85 (juveniles), collected April 1964 by the Departamento de Zoologia (DZ) expedition.

Diagnosis.—The new species is similar to *B. jararaca* (Fig. 4A, Table 1), and is distinguished from this latter species by the combination of characters listed below (*B.*

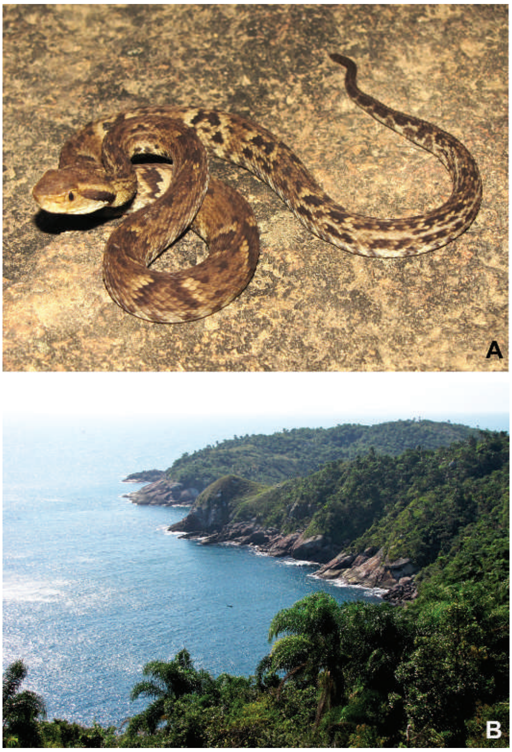

FIG. 4.—*Bothrops otavioi* sp. nov., adult male photographed alive (holotype IBSP 78572; A), and the Atlantic Forest (B) that covers most of the Vitória Island. Photographs by Fernanda Centeno.

jararaca in parentheses). Smaller adult size in males: range SVL = 388–475 mm, *n* = 4 (versus range SVL = 635–1000 mm, *n* = 46); few and larger scales at the intersupraocular row: range = three–six scales (versus range five–nine scales); lower number of ventral scales in males: 182–190 (versus 200–211); lower number of ventral scales in females: 185–192 (versus 190–218); lower number of subcaudal scales in males: 54–61 (versus 62–72); lower number of anterior cephalic scales, generally rounded and with no or feeble keels: range = 20–44 (versus range 27–61, generally elongate and distinctly keeled). The hemipenis of *B. otavioi* sp. nov. (Fig. 5A) has few diminutive ossified spines bordering calyces and they are restricted to the basal region of the capitulum (versus many small ossified spines reaching the medial region of the capitulum, Fig. 5B); intersulcar region nude (versus intrasulcar region with small ossified spines). *Bothrops otavioi* sp. nov. is further distinguished from *B. jararaca* by the absence of diminutive ossified spines from the sulcus spermaticus to the base of the calyces.

From *B. alcatraz*, another small, island-dwelling species, *B. otavioi* sp. nov., can be distinguished by its lower number of intersupraocular scales: three–six (versus six–eight scales); higher number of ventral scales in males: 182–190 (versus 173–182); higher number of ventral scales in females: 185–192 (versus 175–186); higher number of subcaudal scales in males: 54–61 (versus 47–54); rela-

TABLE 1.—Comparison of selected measurements and scale counts for specimens of *Bothrops otavioi* sp. nov., *B. alcatraz*, mainland specimens of *B. jararaca*, and *B. insularis*. Measurements in millimeters, number of specimens in parentheses.

	*B. otavioi*sp. nov.	*B. alcatraz*[a]	*B. jararaca*	*B. insularis*[b]
Snout–vent length adult males	388–475 (4)	365–462 (10)	635-1000 (46)	393–788 (94)
Snout–vent length females	391–692 (8)	365–505 (14)	897-1257 (29)	332–882 (106)
Ventrals in males	182–190 (10)	173–182 (10)	200–211 (15)	171–188 (94)
Ventrals in females	185–192 (11)	175–186 (14)	190–218 (20)	176–195 (106)
Subcaudals in males	54–61 (8)	47–54 (10)	62–72 (15)	55–65 (94)
Subcaudals in females	48–54 (10)	45–52 (14)	53–68 (19)	48–59 (106)
Midbody scale rows	22–25 (17)	22–26 (24)	21–27 (34)	23–25 (200)
Intersupraocular row	3–6 (21)	6–8 (24)	5–9 (34)	7–9 (203)
Anterior cephalics	20–44 (20)	31–48 (24)	27–61 (16)	47–64 (10)
Infralabials	10/10 (5); 10/11 (3); 11/10 (4); 11/11 (5); 11/12 (2); 12/11 (1)	10/10 (19); 10/11 (4); 9/10 (1)	10/10 (6); 10/11 (5); 9/11 (1); 11/11 (15); 11/12 (14); 12/12 (2); 12/10 (1)	10/11 (1); 11/10 (3); 11/11 (6)

[a] Data from Marques et al. (2002).

[b] Amaral (1921).

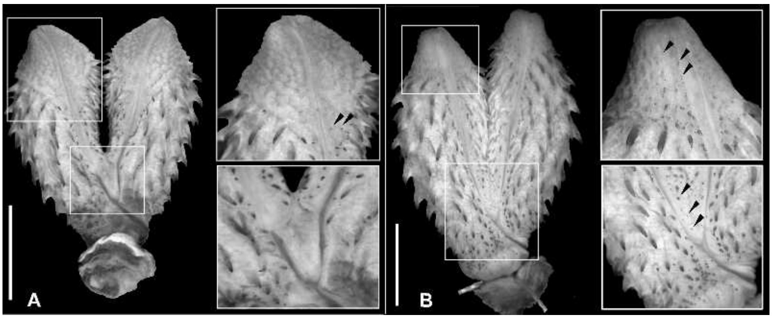

FIG. 5.—Hemipenes in sulcate views: (A) *Bothrops otavioi* sp. nov. (paratype MZUSP 3952); arrows in upper right inset show the small ossified spines only in the basal portion of *capitulum*; lower right inset shows absence of spines in the intrasulcar region; (B) *Bothrops jararaca* (ZUEC 1052) from Ubatuba; arrows in upper right inset show the small ossified spines at the medial portion of *capitulum*; in lower right inset arrows show small ossified spines at intrasulcar region. Line = 5 mm.

tively longer tail in males (ratio TL/SVL ± SD): 0.169 ± 0.010 (versus 0.161 ± 0.007) and in females: 0.146 ± 0.016 (versus 0.138 ± 0.011); relatively longer head in males (ratio HL/TR): 0.057 ± 0.004 (versus 0.053 ± 0.003) and females: 0.061 ± 0.008 (versus 0.055 ± 0.003). *Bothrops otavioi* sp. nov. is distinguished from *B. insularis*, a larger island-dwelling species of the *B. jararaca* group, mainly by its brownish color pattern (pale or yellowish in *B. insularis*), lower number of intersupraocular scales: 3–6 (versus 7–9), lower number of anterior cephalic scales: 20–44 (versus 47–64), and smaller adult size in males: SVL = 388–475 mm (versus 393–788 mm). The new species overlaps with *B. insularis* in number of ventral scales: 182–190 (versus 171–188) in males, and 185–192 (versus 176–195) in females; and subcaudal scales: 54–61 (versus 55–65) in males, and 48–54 (versus 48–59) in females.

Regarding haplotype genealogy, *Bothrops otavioi* sp. nov. is nested within the northern clade of *Bothrops jararaca* group sensu Grazziotin et al. (2006; Fig. 6). The new species has the most common *cytb* haplotype within the northern clade of *B. jararaca* group, sharing the same sequence with populations from Alcatrazes Island, northern São Paulo, and the continental coastal slope to lowlands (Fig. 6).

Description of holotype.—Adult male (Figs. 2, 3, and 4A), preserved in ethanol with right hemipenis partially everted; SVL 391 mm; TL 65 mm (7% of total length); head length 20.4 mm; head width 15.5 mm; mass 27 g (preserved). Rostral scute 2.7 mm wide and 3 mm high; nasals divided anterior and posterior to nostril; loreal single; 0/0 prefoveals; 1/2 postfoveals; prelacunal fused with second supralabial in both sides of head; 2/2 preoculars; 2/2 postoculars; 8/8 supralabials; 3/4 interoculabials; 6/5 circumorbitals; 6/6 temporals; 11/11 infralabials, with the first pair contacting each other posteriorly; mental longer than broad, contacting anteriorly the first three infralabial on each side; five gulars between chin shield and first ventral scale; five rows of gulars separating first ventral scales from infralabials; 2/2 canthals; 7 posterior intercanthals; 3 intersupraoculars (middle scale larger than longer); 39 cephalic scales (scales above top of head + intercanthals scales) with no or feeble keels; 23/23/19 dorsals; 186 ventrals; cloacal scute single; 56 divided subcaudals. Posterior cephalic scales longer than wide and strongly keeled; intersupralabials scales rounded, smooth and weakly keeled; temporal scales keeled; internasals, canthals and supraoculars smooth.

In life, the coloration of the holotype was as follows (Fig. 4A): Ground body color brownish on dorsal surface; 10/9 lateral trapezoidal markings (saddles) dark brown with well-defined borders, weakly white-edged, opposite and alternate to each other on each side of the dorsum. Dorsum of head grayish brown, with seven distinctive small blotches between occipital–temporal portion and neck. Postorbital stripe extends from behind eye to below

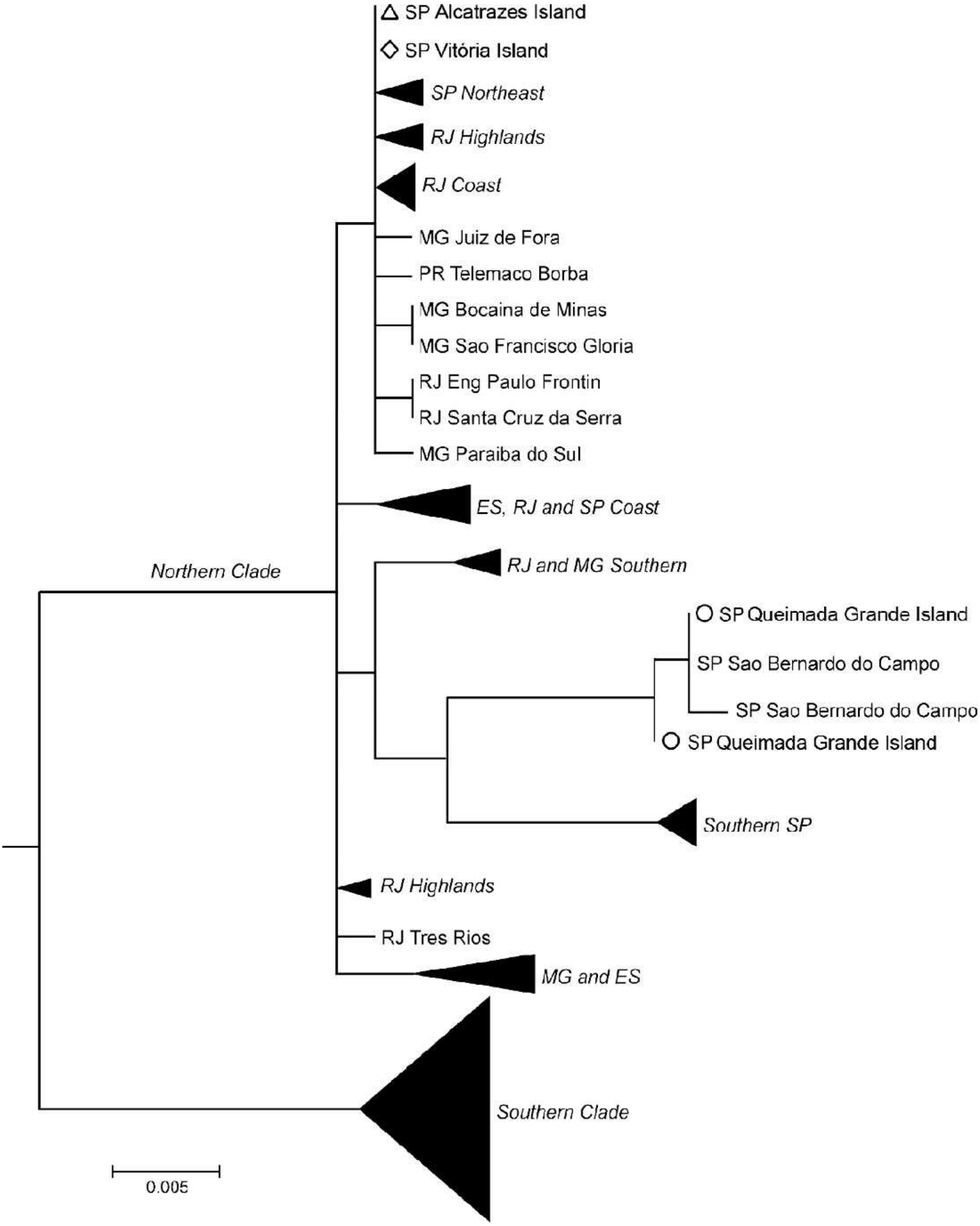

FIG. 6.—Mitochondrial genealogy based on 172 sequences of cytochrome b for *Bothrops jararaca* group. Triangle indicates sequences for *B. alcatraz*; diamond indicates sequence for *B. otavioi* sp. nov.; circles indicates sequences for *B. insularis*; names without shapes are sequences of continental populations of *B. jararaca*. Acronyms for Brazilian states are: SP = São Paulo; RJ = Rio de Janeiro; MG = Minas Gerais; ES = Espírito Santo; and PR = Paraná. Some sequences are grouped and represented as solid triangles, keeping the proportion of sequence frequencies (see Grazziotin et al., 2006, for a full description of the grouped clades).

angle of jaw. Tail brownish dorsally with small dark brown lateral blotches; subcaudals speckled on anterior portion and cream colored posteriorly. Venter speckled irregularly light-yellowish and grayish, covering anal scute and reaching all subcaudal scales.

Variation.—Dorsum brownish, brownish gray, grayish, or dark-olive brown in preservative; lateral trapezoidal markings (saddles) placed opposite each other, partly or completely alternate, with or without well-defined weak white edge. Five specimens (IBSP 18870, IBSP 18874, IBSP 18875, IBSP 78572, and ZUEC 3551) with dark-brown blotches between occipital–temporal region and neck.

Hemipenis.—Bilobed, subcylindrical, bicalyculate, and bicapitate; sulcus spermaticus extending to tip of each lobe (Fig. 5A). Intrasulcar region (sensu Zaher, 1999) without ossified spines; sulcus spermaticus bordered by very few small ossified spines, from the crotch up to calyces. Those small spines are restricted at the basal portion of capitulum.

Sexual dimorphism.—Adult males with relatively longer tails than females ($F_{1,18} = 8.88$, $P = 0.009$). Females with relatively longer heads than males ($F_{1,18} = 4.58$, $P = 0.04$).

Etymology.—The specific epithet *otavioi* honors our friend and colleague Otavio A. V. Marques, a prominent herpetologist at the Instituto Butantan, for his great contribution to the study of natural history and conservation of Brazilian snakes. We suggest the standard English name "Vitória's Lancehead" for the new species.

Distribution.—The new species is known only from the type locality, Vitória Island (23°44′44″ to 23°45′40″S and 45°01′01″ to 45°00′27″W), Ilhabela Archipelago, Brazil. In this archipelago, Vitória is the easternmost island, located ca. 23 km east of São Sebastião Island, and ca. 30 km southeast of Ubatuba, northern coast of São Paulo (Fig. 1), southeastern Brazil. Vitória Island has about 220 ha, with a maximum elevation of ca. 200 m above sea level and with the predominant vegetation being Atlantic forest (Fig. 4B).

Natural History.—The holotype of *Bothrops otavioi* sp. nov. was found active at night, moving on the ground (T.H. Condez, personal communication).Three adult individuals of the new species (IBSP 18871, IBSP 18877, and IBSP 18880) had small hylid frogs in their guts. All specimens of the new species examined that still retained their color pattern had dark tail tips ($n = 30$), which is clearly seen in live specimens (Fig. 4A). Four preserved individuals (IBSP 18867, IBSP 18875, IBSP 18878, and IBSP 18882) out of 21 (19%) had injuries and mutilations on the tail indicated by scars. The only female with follicles or embryos in the oviduct measured 692 mm SVL (K. Kasperoviczus, personal communication). The smallest mature male (with opaque efferent ducts and enlarged testes) measured 388 mm SVL.

Discussion

Three insular species of the *Bothrops jararaca* group are known to date: *B. alcatraz*, *B. insularis*, and *B. otavioi* sp. nov., all of them closely related to the adjacent mainland populations of *B. jararaca* (Marques et al., 2002; this study). Our molecular results corroborated the expectation of a rapid differentiation process for *B. otavioi* sp. nov. As shown by Grazziotin et al. (2006), the haplotype phylogeny of *cytb* indicates that insular species of the *B. jararaca* group are nested inside the mainland diversity of *B. jararaca*. Two evolutionary scenarios can explain such a genealogy: haplotype introgression by recent gene flow between the continental and insular populations, and incomplete lineage sorting by sharing a recent common ancestral population. There is no published study or museum voucher specimen showing evidence of migration of insular species of *B. jararaca* group to the continent, and there is no evidence of migration of the mainland *B. jararaca* to the islands (Bérnils, 2009). If such dispersion events do actually occur, they possibly are very rare, as naturalists have explored the coast of São Paulo state for more than three centuries without reporting such events.

Actually, a gene genealogy pattern such as that found here is the most expected outcome when differentiation involves small populations isolated from a larger ancestral one, as usually occurs with island colonization (Avise, 2000; Funk and Omland, 2003). These situations are even more plausible if the mainland population is very large, has geographic structure, or a short time has passed since isolation (Johnson et al., 2000). All these features are very likely present in the process of insularization experienced by the island species of the *B. jararaca* group.

Grazziotin et al. (2006) discussed the taxonomic issues associated with the possible paraphyly of *B. jararaca*, and concluded that the mitochondrial gene tree probably conflicts with the species tree as a direct result of the speciation process in the *B. jararaca* group. Gene trees conflicting with species trees is a well-known subject in the evolutionary literature (Pamilo and Nei, 1988; Hudson, 1992; Maddison, 1997; Knowles and Carstens, 2007), and an increasingly common outcome of genomics and multilocus analyses (Degnan and Rosenberg, 2009). Any approach to delimit species based exclusively on gene-tree topologies will occasionally fail to enclose all discordances among genealogies (Edwards, 2008). Therefore, our definition of species is not based on reciprocal monophyly of haplotype genealogies. As argued by Kizirian and Donnelly (2007), the application of reciprocal monophyly criterion cannot embrace all recognized biological diversity, because only after sufficient haplotype extinction will the lineage sorting be complete and the species be recovered as monophyletic for all gene genealogies. Consequently, we based the description of *B. otavioi* sp. nov. on morphological characters and the "unique combination of characters states" that makes this population diagnosable (Davis and Nixon, 1992). We use the mitochondrial genealogy only to infer the underlying speciation process. Thus, we emphasize the limited occurrence of the new species on a relatively small and isolated island and the exclusive phenotypic characters, which allowed us to distinguish it from other congeners. Additionally, we assume that the new species represents a lineage that has been evolving separately from other mainland and insular populations of the *B. jararaca* complex, and therefore has its own evolutionary history, as also inferred for the related island species *B. alcatraz* and *B. insularis*.

Currently, the *B. jararaca* group comprises at least four species, of which three are island species. Plausibly, the isolation of each island species from the continental ancestor population occurred at approximately the same time and was caused by the same or similar vicariant process. The oscillations of the sea level during the Pleistocene, of which the last known rise in sea level occurred around the end of the last glaciations period, between 20 and 11 thousand years ago, is the most accepted hypothesis and can explain the isolation and evolutionary history of these island species (Martin et al., 1986; Rodrigues, 1990; Marques et al., 2002). The occurrence of those insular forms could be also related to dispersal after the isolation of the continental islands. Although both vicariance and dispersal are plausible mechanisms for the isolation of *Bothrops* spp. in continental islands of the Atlantic forest, vicariance has been the preferred hypothesis for speciation on Brazilian continental islands, because it can explain the simultaneous speciation patterns based on fewer phenomena, and can even explain the speciation of amphibians (Brasileiro et al., 2007a,b), which are much more sensitive to desiccation than are reptiles.

Bothrops otavioi sp. nov. possibly shares several ecological attributes with *B. jararaca* continental populations, such as forest-dwelling habit and mostly nocturnal foraging on the ground (Sazima, 1992). The stouter and shorter body of the new species is shared with *B. alcatraz*, another relatively small, island-dwelling species of the *B. jararaca* group (Marques et al., 2002). It is tempting to speculate that these two small species traded size for robustness.

Frogs are the most common prey animal found in the guts of mainland juveniles of *B. jararaca*, which may catch these prey with caudal luring, a behavior recently described for juvenile *B. insularis* as well (Sazima, 1991; Hartmann et al., 2003; Marques and Sazima, 2004; Sazima, 2006; Andrade et al., 2010). Most mainland juveniles of *B. jararaca* have a whitish or yellowish tail tip that contrasts with the darker background body color, whereas the island-dwelling *B. insularis* display a brownish tail tip that contrasts with the lighter background color (Sazima, 1991; Sazima, 2006; Andrade et al., 2010). Juveniles of *B. jararaca* that have brownish tail tips also lure frogs successfully (Sazima, 1991, 1992). The specimens of the new species that had injuries on the tail and frogs in the gut lend support to the idea that *B. otavioi* sp. nov. lures prey with a dark-brown tail tip, as recently suggested for both juveniles and adults of the dark-tailed, island-dwelling *B. insularis* (Andrade et al., 2010; see also Martins et al., 2002).

Although nested within the northern clade of *B. jararaca*, no examined individual of *B. otavioi* sp. nov. or *B. alcatraz* (which also feeds on ectothermic prey) had light-colored tail tip, as recorded for juveniles of *B. jararaca* (Sazima, 1991, 1992). We speculate that the absence of yellowish or whitish tail tip in both Alcatraz's and Vitória's lanceheads may be explained by a few individuals of the ancestor population having a dark-brown tail tip and this character becoming fixed in both of these island-dwelling species, similarly to the "hemiclitoris" found in all females of *B. insularis* and rarely in females of the mainland *B. jararaca* (F.E. Barbo, F.G. Grazziotin, I. Sazima, M. Martins, and R.J. Sawaya, personal observations).

Sexual maturity of *B. otavioi* sp. nov. is attained at a relatively small size (388 mm SVL in males, and 692 mm SVL in females), as also found for the island-dwelling *B. alcatraz*, whose males attain sexual maturity at 365 mm SVL and females at 477 mm SVL (Marques et al., 2002). Mainland adults of *B. jararaca* attain sexual maturity at about 650 mm SVL in males and 750 mm SVL in females (Sazima, 1992; Janeiro-Cinquini et al., 1993). For *B. insularis*, the smallest mature female was 505 mm SVL (K. Kasperoviczus, personal communication). Thus, *B. otavioi* sp. nov. may be a second instance of a paedomorphic species within the *B. jararaca* group, as already suggested for *B. alcatraz* (Marques et al., 2002).

The new species should be included in national and global red lists as critically endangered (CR B1a,b[iii], following IUCN, 2001) because its extent of occurrence (ca. 2.2 km^2) is less than 100 km^2, it is known from a single location ("an area in which a single threatening event can rapidly affect all individuals of the taxon present"; IUCN, 2001), and there is a projected continuing decline in the quality of its habitat due to increasing human settlement. The two other island-dwelling species of the *B. jararaca* group, *B. alcatraz* and *B. insularis*, also are critically endangered in the Brazilian and the global red lists (Marques et al., 2004a,b; Machado et al., 2005).

Acknowledgments.—We are grateful to F. Centeno and T. Condez for the collected material, and for photographs of a live specimen of the new species and the island; V. Germano for help in the laboratory; J.C. Arredondo and L. Oliveira for help with photographs of the hemipenes and data collection; K. Kasperoviczus for data on reproduction of insular species of *Bothrops*; H. Zaher and C. Mello for help and access to the MZUSP specimens; O.A.V. Marques for valuable suggestions about insular species of *Bothrops*; R. Nunes for data on mainland *B. jararaca* populations. FEB and FGG thank the FAPESP for scholarships (08/50068–2 and 07/52781–5, respectively). RJS and MM thank the CNPq for research fellowships. RJS thanks the FAPESP for grants under the project "Diversidade, Distribuição e Conservação da Herpetofauna do Estado de São Paulo" (08/54472–2), and INCTTOX PROGRAM of CNPq and FAPESP. MM thanks FAPESP for a research grant (10/50146–3). IS thanks the CNPq for earlier financial support. We also thank the FAPESP for grants under the project "Origem e Evolução das Serpentes e a sua Diversificação na Região Neotropical: Uma Abordagem Multidisciplinar" (2011/50206–9). Permit for collection of the holotype was provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under number 14858–2 to RJS and FEB.

Literature Cited

Amaral, A. 1921. Contribuição para o conhecimento dos ophidios do Brasil. Parte I. Quatro novas espécies de serpentes Brasileiras. Anexos das Memórias do Instituto Butantan 1:1–37.

Andrade, D.V., O.A.V. Marques, R.S.B. Gavira, F.E. Barbo, R.L. Zacariotti, and I. Sazima. 2010. Tail luring by the golden lancehead (*Bothrops insularis*), an island endemic from southeastern Brazil. South American Journal of Herpetology 5:175–180.

Avise, L.C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, USA.

Bérnils, R.S. 2009. Composição e Padrões de Distribuição de Caenophidia (Squamata, Serpentes) das Serras Atlânticas e Planaltos do Sudeste da América do Sul. Ph.D. Dissertation, Universidade Federal do Rio de Janeiro, Brazil.

Brasileiro, C.A., C.F.B. Haddad, R.J. Sawaya, and M. Martins. 2007a. A new and threatened species of *Scinax* (Anura: Hylidae) from Queimada Grande Island, southeastern Brazil. Zootaxa 1391:47–55.

Brasileiro, C.A., C.F.B. Haddad, R.J. Sawaya, and I. Sazima. 2007b. A new and threatened island-dwelling species of *Cycloramphus* (Anura: Cicloramphidae) from southeastern Brazil. Herpetologica 63:501–510.

Campbell, J.A. 1985. A new species of highland pitviper of the genus *Bothrops* from southern Mexico. Journal of Herpetology 19:48–54.

Campbell, J.A., and W.W. Lamar. 1989. The venomous reptiles of Latin America. Cornell University Press, USA.

Campbell, J.A., and W.W. Lamar. 2004. The venomous reptiles of the Western hemisphere. Comstock Publishing, USA.

Carrasco, P.A., G.C. Leynaud, and G.J. Scrocchi. 2010. Redescription of the southernmost snake species, *Bothrops ammodytoides* (Serpentes: Viperidae: Crotalinae). Amphibia–Reptilia 31:323–338.

Carrasco, P.A., C.I. Mattoni, G.C. Leynaud, and G.J. Scrocchi. 2012. Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). Zoologica Scripta 41:1–15.

Cicchi, P.J.P., M.A. Sena, D.M. Peccinini-Seale, and M.R. Duarte. 2007. Snakes from coastal islands of State of São Paulo, Southeastern Brazil. Biota Neotropica 17:227–240.

Davis, J.I., and K.C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41:421–435.

Degnan, J.H., and N.A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology and Evolution 24:332–340.

Duarte, M.R., G. Puorto, and F.L. Franco. 1995. A biological survey of the pitviper *Bothrops insularis* Amaral (Serpentes, Viperidae): An endemic and threatened offshore island snake of southeastern Brazil. Studies on Neotropical Fauna and Environment 30:1–13.

Edwards, S.V. 2008. Is a new and general theory of molecular systematics emerging? Evolution 6:1–19.

Fenwick, A.M., R.L. Gutberlet, Jr., J.A. Evans, and C.L. Parkinson. 2009. Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (Serpentes: Viperidae). Zoological Journal of the Linnean Society 156:617–640.

Funk, D.J., and K.E. Omland. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology and Systematics 34:397–423.

Garman, S. 1881. New and little-known reptiles and fishes of museum collections. Bulletin of the Museum of Comparative Zoology 8:85.

Grazziotin, F.G., M. Monzel, S. Echeverrigaray, and S.L. Bonatto. 2006. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): Past fragmentation and island colonization in the Brazilian Atlantic Forest. Molecular Ecology 1–14.

Hartmann, P.A., M.T. Hartmann, and L.O.M. Giasson. 2003. Uso do hábitat e alimentação em juvenis de *Bothrops jararaca* (Serpentes, Viperidae) na Mata Atlântica do sudeste do Brasil. Phyllomedusa 2:35–41.

Hudson, R.R. 1992. Gene trees, species trees and the segregation of ancestral alleles. Genetics 131:509–512.

IUCN. 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Switzerland.

Janeiro-Cinquini, T.R.F., F.F. Leinz, and E.C. Farias. 1993. Ovarian cycle of the *Bothrops jararaca*. Memórias do Instituto Butantan 55:33–36.

Johnson, K.P., F.R. Adler, and J.L. Cherry. 2000. Genetic and phylogenetic consequences of island biogeography. Evolution 54:387–396.

Kizirian, D., and M.A. Donnelly. 2007. The criterion of reciprocal monophyly and classification of nested diversity at the species level. Molecular Phylogenetics and Evolution 32:1072–1076.

Knowles, L.L., and B.C. Carstens. 2007. Delimiting species without monophyletic gene trees. Systematic Biology 56:887–895.

Larkin, M.A, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

Machado, A.B.M, C.S. Martins, and G.M. Drummond (Eds.). 2005. Lista da Fauna Brasileira Ameaçada de Extinção: Incluindo as Espécies Quase Ameaçadas e Deficientes em Dados. Fundação Biodiversitas, Brazil.

Maddison, W.P. 1997. Gene trees in species trees. Systematic Biology 46:523–536.

Marques, O.A.V., and I. Sazima. 2004. História natural dos répteis da Estação Ecológica Juréia-Itatins. Pp. 257–277 in O.A.V. Marques and W. Duleba (Eds.), Estação Ecológica Juréia-Itatins: Ambiente Físico, Flora e Fauna. Holos Editora, Brazil.

Marques, O.A.V., M. Martins, and I. Sazima. 2002. A new species of pitviper from Brazil, with comments on evolutionary biology and conservation of the *Bothrops jararaca* group. Herpetologica 58:303–312.

Marques, O.A.V., M. Martins, and I. Sazima. 2004a. *Bothropoides alcatraz*. In IUCN Red List of Threatened Species. Version 2011.1. Available at http://www.iucnredlist.org.

Marques, O.A.V., M. Martins, and I. Sazima. 2004b. Bothropoides insularis. In IUCN Red List of Threatened Species. Version 2011.1. Available at http://www.iucnredlist.org.

Martin, L., N.A. Mörner, J.M. Flexor, and K. Suguio. 1986. Fundamentos e reconstrução de antigos níveis marinhos do Quaternário. Boletim do Instituto de Geociências, Publicação Especial 4:1–161.

Martins, M., O.A.V. Marques, and I. Sazima. 2002. Ecological and phylogenetic correlates of feeding habits in Neotropical pitvipers of the genus *Bothrops*. Pp. 307–328 in G. Schuett, M. Höggren, and H. W. Greene (Eds.), Biology of the Vipers, Eagle Mountain Publishing, USA.

Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5:568–583.

Pook, C.E., W. Wüster, and R.S. Thorpe. 2000. Historical biogeography of the western rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. Molecular Phylogenetics and Evolution 15:269–282.

Rodrigues, M.T. 1990. Os lagartos da floresta Atlântica distribuição atual e pretérita e suas implicações para estudos futuros. Pp. 404–410 in II Simpósio Sobre Ecossistemas da Costa Sul Brasileira: Estrutura, Manejo e Função. Academia de Ciências do Estado de São Paulo, Brazil.

Salomão, M.G., W. Wüster, and R.S. Thorpe, and BBBSP (Butantan–British *Bothrops* Systematics Project.). 1997. DNA evolution of South American pit vipers of the genus *Bothrops*. Pp. 89–98 in R. S. Thorpe, W. Wüster, and A. Malhotra (Eds.), Venomous Snakes: Ecology, Evolution and Snakebite. Clarendon Press, UK.

Salomão, M.G., W. Wüster, and R.S. Thorpe, and BBBSP (Butantan–British *Bothrops* Systematics Project). 1999. MtDNA Phylogeny of neotropical pitvipers of the genus *Bothrops* (Squamata: Serpentes: Viperidae). Kaupia: Darmstädter Beiträgezur Naturgeschichte 8:127–134.

Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual (3rd Ed.). Cold Spring Harbor Laboratory Press, USA.

Sazima, I. 1991. Caudal luring in two Neotropical pitvipers, *Bothrops jararaca* and *B. jararacussu*. Copeia 1991:245–248.

Sazima, I. 1992. Natural history of the jararaca pitviper, *Bothrops jararaca*, in southeastern Brazil. Pp. 199–216 in J. A. Campbell, and E. D. Brodie (Eds.). Biology of Pitvipers. Selva, USA.

Sazima, I. 2006. Theatrical frogs and crafty snakes: Predation of visually-displaying frogs by tail luring and ambushing pitvipers. Aqua, International Journal of Ichthyology 11:117–124.

Statsoft. 1995. Statistica for Windows, Release 5.0. Statsoft, Inc., USA.

Statsoft. 2001. Statistica for Windows, Release 6.0. Statsoft, Inc., USA.

Swofford, D.L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, USA.

Wüster, W., M.G. Salomão, J.A. Quijada-Mascareñas, and R.S. Thorpe, and BBBSP (Butantan–British *Bothrops* Systematics Project.) . 2002. Origins and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. Pp. 111–128 in G. Schuett, M. Höggren, and H. W. Greene (Eds.). Biology of the Vipers. Eagle Mountain Publishing, LC, USA.

Zaher, H. 1999. Hemipenial morphology of the South American xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. Bulletin of the American Museum of Natural History 240:1–168.

Zar, J.H. 1996. Biostatistical Analysis. Prentice–Hall Inc., USA.

Accepted: 16 March 2012
Associate Editor: Christopher Raxworthy

APPENDIX

Specimens Examined

Bothrops alcatraz.—**São Paulo state:** São Sebastião: Ilha dos Alcatrazes (IBSP 13031, 13126, 13183, 55578, 56133).

Bothrops insularis.— **São Paulo state:** Itanhaém, Ilha da Queimada Grande (IBSP 667–68, 1254, 1884, 1918, 1928, 1953, 1971, 1984, 1992).

Bothrops jararaca.— **São Paulo state:** Bertioga (IBSP 64230–31); Cananéia (IBSP 16601, 19548, 22261, 23018); Capão Bonito (ZUEC 2039); Caraguatatuba (IBSP 26730–35); Cubatão (IBSP 11966, 18674, 21670, 62072, 67182); Cunha (ZUEC 1564); Guapiara (IBSP 64461); Guarujá (IBSP 55524, 55863, 55864, 64763–64); Iguape (IBSP 1076, 24692, 55196, 56259, 57800, 57802, 57805, 57808, 57811, 57817, 57840–41, 57856, 57888, 57896–97, 57903–04, 57908, 57927–28, 57940–41, 57967, 57989, 57995, 58001, 58009, 58035, 58086, 58108, 58129–30, 58132, 58134, 58145, 58156, 58166–67, 58264–65, 61815, 64090, 64462, 64683, 66473, 66665, 66768); Ilhabela (IBSP 75975–90); Itanhaém (IBSP 11177–78, 11557, 11760–61, 12606, 18548, 18723, 18918, 19935–36, 20879–82, 53102, 56360, 56475, 60669–70, 61821, 66582, 66746); Itariri (IBSP 1105); Miracatu (IBSP 28965, 28969, 55993, 56835, 57704); Mongaguá (IBSP 56997–57004, 57247, 61945, 62889); Pedro de Toledo (IBSP 18777, 18917, 18933, 18957–58, 24065–66, 61383, 61407, 67281); Peruíbe (IBSP 12581, 19036, 20727, 55195, 57790, 57792, 57866, 57869, 57871, 57880, 57882, 57920, 57942, 57945, 57948–49, 57953, 58002, 58014, 58077, 58088, 58090, 64658, 66918); Registro (IBSP 18281, 23321–23, 53916, 61205–07); Ribeirão Branco (ZUEC 1746, 1770); Santo André, district of Paranapiacaba (ZUEC 862); Santos (IBSP 10459, 18848, 19501, 21684–88, 61373); São José do Barreiro (ZUEC 861); São Sebastião (MZUSP 1403–04, 2267); São Sebastião, Maresias beach (MZUSP 12351); São Sebastião, Juquehy beach (MZUSP 12737, 12819); São Sebastião, Barra do Una (MZUSP 13167); São Sebastião, Engenho beach (MZUSP 15137); São Vicente (IBSP 14547, 15840, 18722, 57403); Sete Barras (IBSP 56005–07); Ubatuba (IBSP 69042, 69047–48, 69055–57, 69060–62, 69064, ZUEC 1052 [hemipenis])