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FRONT COVER: *Pseudablabes agassizii* swallowing a spider (R. Bertani)

A NEW SPECIES OF *MABUYA* (REPTILIA, SQUAMATA, SCINCIDAE) FROM THE CARIBBEAN ISLAND OF SAN ANDRÉS, WITH A NEW INTERPRETATION OF NUCHAL SCALES: A CHARACTER OF TAXONOMIC IMPORTANCE

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A new species of *Mabuya* lizard from the isolated Caribbean island of San Andrés is described. This species is closely related to *Mabuya pergravis* Barbour, 1921, another poorly known species from Providencia Island, 87 km NNE of San Andrés. Unfortunately this new species, known from a single specimen, is now probably extinct. It differs from *M. pergravis* in many morphological characters such as a smaller size and very different patterns of coloration, but most importantly in the presence of a very high number of nuchal scales. A new definition of this last character, which is of systematic importance in the genus *Mabuya*, is also given and discussed.

Key words: insular endemism, *Mabuya berengeriae*, San Andrés Archipelago, skink

INTRODUCTION

The San Andrés Archipelago is made up of two major isolated islands in the south-west Caribbean Sea. San Andrés (St. Andrew) and Providencia (Old Providence). These small Colombian islands are situated 197 km and 235 km respectively from the Nicaraguan coasts. Providencia lies at 87 km north–north-east of San Andrés. The latter is situated at 365 km in the north off the coast of Panamá, while Providencia is at 626 km from Jamaica, its nearest neighbour among the Antillean Islands (Fig. 1). Despite their proximity to each other and relationship to the continental landmass, San Andrés and Providencia are thought to have been uplifted independently in the early Tertiary and to have never had a land bridge either between them, or between the mainland (Parsons, 1956). San Andrés is made of uplifted Tertiary and Pleistocene rocks, and Holocene terrestrial and beach deposits (Geister, 1975).

Mabuya pergravis is an endemic species from the Island of Providencia. This species, described in 1921 by Barbour, was recognized by Dunn (1936, 1945) as a full

species, then later regarded as a subspecies of *M. mabouya* (Dunn & Saxe, 1950; Valdivieso & Tamsitt, 1963). However, I consider *M. pergravis* as a species in its own right, even though it has some characteristics in common with the two other, widely distributed Caribbean species, *M. mabouya* and *M. sloanii* (considered by Dunn & Saxe [1950] as two populations of the same species *M. mabouya*, the first one being native from the southern Lesser Antilles and the second one from both the northern Lesser Antilles and Greater Antilles). *Mabuya pergravis* differs from its geographical closest neighbour, *M. sloanii*, in having a longer snout, a larger size and in the absence of two dorsal stripes. It differs also from *M. mabouya* in having four supraoculars (versus three) and a longer snout (Miralles, 2005), this last point being highly supported by the very wide gap of about 2200 km which separates their respective geographical distributions.

During a systematic revision of the genus *Mabuya*, a specimen (UMMZ 127884, firstly identified as a *M. pergravis* in the collection catalogue of the UMMZ) collected from the Island of San Andrés, was found. The presence of the genus on the island was never mentioned until now. After a comparison with two specimens of *M. pergravis*, USNM 13875 (holotype) and USNM 76947, it became clear the specimen from San Andrés does not conform to the species from the adjacent island of Providencia, nor with any other New World *Mabuya*. Even though *M. pergravis* and the specimen from San Andrés share some characteristics, such as a very long and pointed snout, some major characteristics distinguish the two forms easily, such as differences in scalation, size and colour patterns (Fig. 2). Given that (1) San Andrés island was never in contact with Providencia (Parsons, 1956), and is 87 km away from it; and (2) the specimen from San Andrés differs in many morphological characteristics from *Mabuya pergravis*, I hypothesize that the specimen UMMZ 127884 belongs to a species unknown to science. This paper describes this new species.

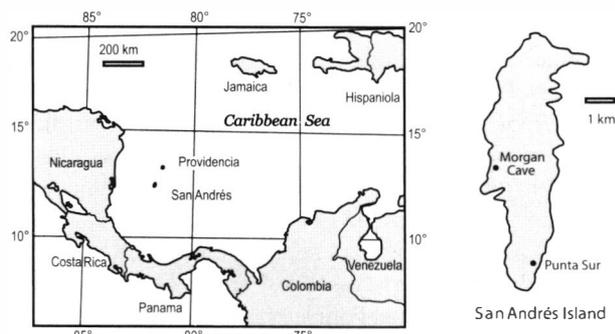


FIG. 1. Localisation of the two Islands of Providencia and San Andrés, with map of San Andrés Island.

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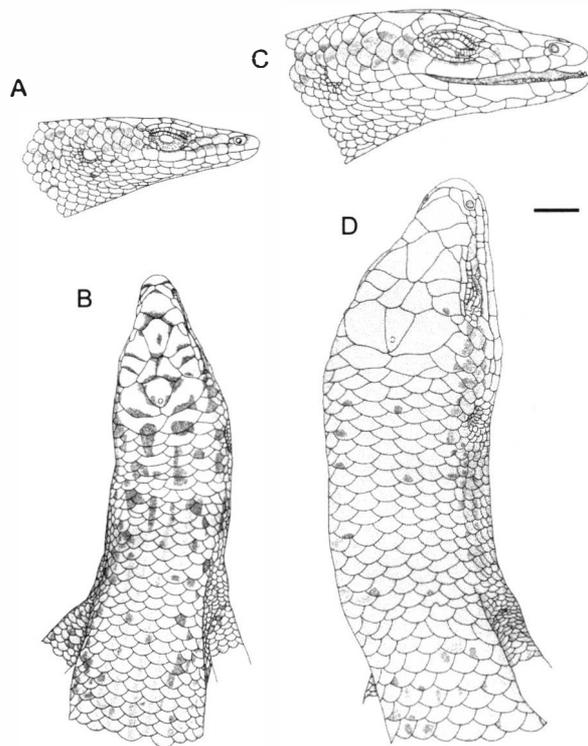


FIG. 2. Drawings of the head of the holotype of *Mabuya berengeriae* (UMMZ 127884). A, lateral view of the head; B, dorsal view of the anterior part of the body; and of the holotype of *Mabuya pergravis* (USNM 13875); C, lateral view of the head; D, dorsal view of the anterior part of the body. Scale bar = 3 mm.

MATERIALS AND METHODS

According to its former definition, *Mabuya* Fitzinger, 1826 was considered as a worldwide distributed genus of tropical Scincidae (with the exclusion of Australia and Pacific islands). However, a recent phylogenetical study (Mausfeld *et al.*, 2002) demonstrated that all Neotropical *Mabuya* species clearly form a monophyletic lineage (except *M. atlantica*, an endemic species from Fernando de Noronha Archipelago off Brazil, belonging to the African lineage, firstly classified by Mausfeld *et al.* (2002) as *Euprepis* Wagler, 1830, then replaced by Bauer (2003) by *Trachylepis* Fitzinger, 1843, in accordance with the rules of the Code of Zoological Nomenclature). Following these authors, I consider *Mabuya* in the present paper as an exclusively Neotropical genus, encompassing Central America, South America, and Caribbean islands.

The specimens examined for this study were preserved in 70% ethanol and deposited at the American Museum of Natural History, New York (AMNH), the Carnegie Museum, Pittsburgh (CM), the Field Museum of Natural History, Chicago (FMNH), the Museo de Historia Natural La Salle of Caracas (MHNLS), the Muséum National d'Histoire Naturelle, Paris (MNHN), the Sam Noble Oklahoma Museum of Natural History, Norman (OMNH), the University of Michigan Museum of Zoology, Ann Arbor (UMMZ) and the National Museum of Natural History, Smithsonian Institution, Washington (USNM).

Drawings were made with a stereomicroscope LEICA MS5 equipped with a camera lucida. The scale nomenclature, scale counts and measurements used in the description of specimen are based after Avila-Pires (1995). New characters of first rank in systematics for the genus *Mabuya*, proposed by Greer & Broadley (2000), and Greer & Nussbaum (2000) were also added to the description. Moreover, a new definition of the nuchal scales is given in the discussion of the present paper.

MABUYA BERENGERAE SP. NOV.

Holotype. UMMZ 127884. An unsexed probably subadult or adult specimen from San Andrés Island (between Morgans Cave and Punta Sur), Departamento de Archipiélago de San Andrés, Colombia [located between 12° 28' and 12° 36'N, and 81° 40' and 81° 44'W], collected on 6 August 1967 by C. F. Walker. The collector identified this specimen as a *M. pergravis*, and wrote accompanying field notes “*basking on leaf three feet above the ground when first seen*” (G. Schneider, pers. comm.).

Diagnosis. *Mabuya berengeriae* is a particularly uncommon species which can be easily distinguished from all other *Mabuya* species, including its closest relative *M. pergravis*, by the following combination of characters: an extremely long, slender and pointed snout, the highest number of enlarged nuchal scales of any known species of *Mabuya* (Table 1), a high number of subdigital lamellae under the fourth toes (19) and a contrasting reticulated pattern on the upper side of the head, the neck and the back, also unique in the genus (Fig. 2B, 3).

Description of the holotype. UMMZ 127884 (Fig 2A-B, 3). Very good state of preservation. Snout-vent length 52.8 mm, tail (regenerated) length 28.8 mm, Head length 11.7 mm. Rostral wider than high. Fore and hind limbs easily touching each other when adpressed against body. A pair of internasals in median contact. A frontonasal, approximately hexagonal, as wide as long, laterally in contact with anterior loreal scale. A pair of quadrilateral prefrontals, separated medially, and in contact with frontonasal, both anterior and posterior loreals, first supraciliary, first and second supraoculars, and frontal. Frontal rhomboid (almost triangular), in contact with all the length of the second supraocular. A pair of frontoparietals, each in contact with frontal, the supraoculars except the first, parietal and interparietal. Interparietal triangular, separated from nuchals by parietals; parietal eye distinct. Parietal overlapping the upper temporal scale. Four supraoculars, the second one being the longest and largest. The posteriormost supraocular in contact with the frontal is the second one. Four supraciliaries, the second the longest. Nostril in posterior part of the nasal. A small postnasal, bordered by supranasal, anterior loreal and first supralabial. An anterior and a posterior loreal, both elongate. A frenal and two pre-suboculars. Lower eyelid undivided, with a transparent disk. A single row of small

TABLE 1. Number of primary and secondary pairs of enlarged nuchals (nuchals I and II) in some species of *Mabuya*. The table is divided in two parts: species without pair of secondary enlarged nuchal (upper part), and species with them (lower part). When specimens had an asymmetric number of nuchals on each side of the nape, the highest number of the two was scored. Data for species followed by an asterisk (*) are entirely or partly based on literature (Dunn & Saxe, 1950; Rebouças-Spieker & Vanzolini, 1990; Avila-Pires, 1995 and Hoge, 1946).

Species	Presence of nuchals I		Number of nuchals II				
	none	1	none	1	2	3	≥4
<i>M. bistrinata</i> (n=23)	-	23	23	-	-	-	-
<i>M. falconensis</i> (n=5)	-	5	5	-	-	-	-
<i>M. frenata</i> (n=6)	-	6	6	-	-	-	-
<i>M. heathi</i> (n=4)	-	4	4	-	-	-	-
<i>M. nigropunctata</i> (n=91)	2	89	90	1	-	-	-
<i>M. mabouya</i> (n=11)	-	11	11	-	-	-	-
<i>M. pergravis</i> (n=20)*	-	20	15	5	-	-	-
<i>M. unimarginata</i> (n=18)	-	18	18	-	-	-	-
<i>M. berengeriae</i> (n=1)	-	1	-	-	-	-	1
<i>M. carvalhoi</i> (n=4)*	-	4	-	-	3	1	-
<i>M. croizati</i> (n=14)	-	14	-	1	8	5	-
<i>M. nigropalmata</i> (n=5)*	-	5	-	-	5	-	-
<i>M. macrorhyncha</i> (n=3)*	-	3	-	3	-	-	-
<i>M. sloanii</i> (n=19)	-	19	-	13	4	1	-

scales across the dorsal edge of the eyelid window. Eight supralabials, the sixth being the widest and forming the lower border of the eyelid. Two pretemporal scales, a single primary temporal, two secondary temporals in contact and three tertiary temporals. Temporal scales imbricate, smooth, cycloid, not distinctly delimited from the scales on the nape and the sides of the neck. Ear-opening relatively small, oval, with undulating anterior margin and smooth posterior margin. Mental scale wider than long, posterior margin straight. Postmental scales and a pair of chin shields in contact with infralabials. First pair of chin shields in contact medially, second pair separated by a smaller cycloid scale. Gulars similar to ventrals. Eight infralabials. One pair of primary nuchal scales, the left one being laterally fused with the left upper tertiary temporal; number of secondary nuchal scales asymmetric: five on the right side, four on the left one (see Discussion). Other scales on nape similar to dorsals. On lateral sides of neck, scales

slightly smaller. Dorsal scales cycloid, imbricate, smooth; 56 in a longitudinal row. Ventrals similar to dorsals; 37 in a longitudinal row. No distinct boundaries between dorsals, laterals and ventrals; 28 scales around midbody. Preanal plate with scales similar to ventral. Scales on tail similar to dorsals, except for its posterior part which is regenerated. Palms and soles light coloured, covered with small tubercles, subequal in size. Both regions delimited by a row of larger and flatter scales. Subdigital lamellae smooth, single, under fourth finger 14 (right) and 15 (left), under fourth toe 19 (on each side). Finger and toes clawed; toes length in the following order: I < II < III = V < IV.

The colour in life is unknown. In preservative, the background colour of the flanks and the upper side of the head, neck, back, legs and tail is bronze, with six very narrow and slightly darker dorsal lines running from the middle of the nape to the base of the tail. Supraciliaries, supranasals and lips are very light cream

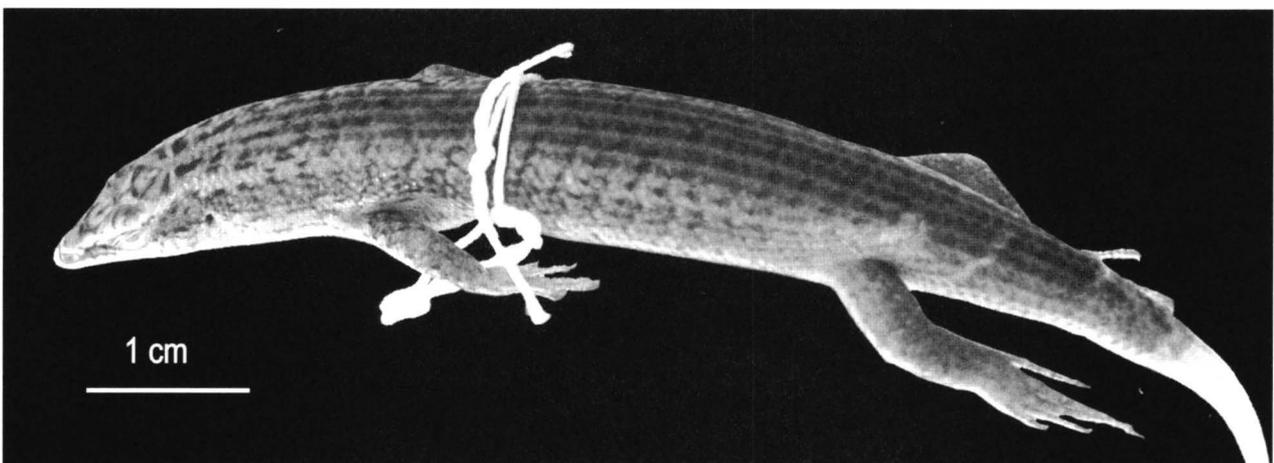


FIG. 3. Photograph of the holotype of *Mabuya berengeriae* (UMMZ 127884). Scale bar = 1 cm.

coloured. A brown lateral strip, itself spotted with darker dots, extends each side from the nostril (loreal, upper third part of the supralabials, around eyes and temporals), along the upper half part of the ear-opening, and continues in a lighter reticulated gradation on the neck to above the arms (width on neck about three scale rows). Three nearly imperceptible light lines are supposed to have been more discernible in the living animal: a vertebral line, running from nape to the tail, and two dorsolateral ones, on each side along the lateral brown strips, from the lateral part of the parietals to above the arms. Many dark brown symmetric spots accentuate posterior sutures of nearly all supracephalic scales, others elongate spots run on the neck. The back, sides and upper side of the legs are spotted with small fused dark dots, forming many transverse narrow stripes. The ventrum, lower side of head, neck, lower side of the limbs and tail are cream-coloured with some grey marks due to the transparency of the ventral skin.

Etymology. The name has been given in honour of my wife Bérengère, for her invaluable support during all my studies.

Remark. It appears quite probable that this species, known from a single specimen, is now extremely rare. For this reason, I avoided the dissection of the holotype that would be necessary to determine its sex.

DISCUSSION

CONSERVATION, ENDEMISM AND BIOGEOGRAPHY

During the seventeenth and eighteenth centuries, San Andrés apparently supported extensive natural stands of "Cedar" (possibly *Cedrela odorata*), which were decimated by early colonists (Emmel, 1975). Most of the island is presently devoted to the cultivation of coconut palms, *Cocos nucifera* (covering the southern half of the island) and Colombian tourism. The human population of San Andrés increased from 17 000 in 1967 (Emmel, 1975) to over 50 000 in 1984 (Johnson, 1988) and is likely growing still. Gonzalez *et al.* (1995) comment on the state of conservation of the original biotopes on San Andrés and Providencia islands "It has been an unequal struggle: so many hectares of Primary forest mainly from San Andrés, have been sacrificed to give space for hundreds of cement tons". Unfortunately, *Mabuya berengerae* is only known from a single specimen caught in 1967. Given the small size of the island, its very damaged ecosystem (Emmel, 1975; Barriga *et al.*, 1985; Johnson, 1988; Gonzalez *et al.*, 1995) and the fact that this specimen is the only one known, it appears highly probable that this species is now endangered, if not extinct. For those reasons, a faunistic survey on this island seems to be urgently needed, in particular for a re-assessment of the current conservation status of *M. berengerae*, but also in a more general perspective, to have an accurate assessment of the degree of endemism on this island and to elaborate preservation strategies to protect it.

Indeed, the San Andrés Archipelago appears to have a unique evolutionary history. Despite the fact that few studies were published on the fauna of San Andrés Island, five endemic taxa of terrestrial vertebrates have been described in addition to *Mabuya berengerae*, from it: three reptiles out of thirteen known from the Archipelago (Valdivieso & Tamsitt, 1963), including a dipsadine snake species, *Coniophanes andresensis* Bailey, 1937, a species of polychrotid (also present on Providencia), *Norops concolor* (Cope, 1862), and a subspecies of gekkonid lizard, *Sphaerodactylus argus andresensis* Dunn & Saxe, 1950; but also two passerines birds including a species of mimid, *Mimus magnirostris* Cory, 1887 and a species of vireonid, *Vireo caribaeus* Bond & Meyer de Schauensee, 1942. On the other hand, no amphibian or mammal species appears to be endemic to the island. So high a degree of endemism in so small an area (about 44 km²) strongly suggests a unique history and a prolonged isolation of this island.

Given the large distance that separates the San Andrés Archipelago from the other Caribbean islands, its biogeographical affinities are not clear. Its original fauna and flora seem to be a mix of both Mesoamerican and Caribbean elements (Riley, 1975; Smith *et al.*, 1989; Gonzalez *et al.*, 1995). Although it seems certain that *M. berengerae* and *M. pergravis* are sibling species (their extremely elongate and pointed snout being the most remarkable synapomorphy of their monophyletic lineage), the origin of this clade on the archipelago is still unresolved. On the basis of their scale characters, Dunn & Saxe (1950) conclude that the *Mabuya* from Providencia seemed to be more closely related to the Lesser Antillean populations than to those from the adjacent mainland. Indeed, *M. pergravis* and *M. berengerae* share with *M. mabouya* (present in the southern Lesser Antilles from Dominica, Guadeloupe, Martinique and Saint Lucia) a slender muzzle and a short lateral strip from snout to above arms. They share also with *M. sloanii* (present in northern Lesser Antilles and in the Great Antilles) the presence of secondary nuchal scales. Contrary to the biogeographical trends based on botanical or entomological studies, no affinities seem to be shared by Mesoamerican species of *Mabuya* and the San Andrés Archipelago's clade.

However, species belonging to the genus *Mabuya* are often very similar and external morphological characters are usually used for studying their taxonomy. Unfortunately, most of these characters could easily result from convergence. For this reason, only a real phylogenetical approach would precisely determine the relationships of this small clade.

WHAT IS A NUCHAL SCALE?

The organisation of the nuchal scales, transversally enlarged dorsal scales of the nape, is a major characteristic in the systematics of the genus *Mabuya*, and more generally in all the lygosomines skinks. Notably, it represents one of the most important diagnostic characters for

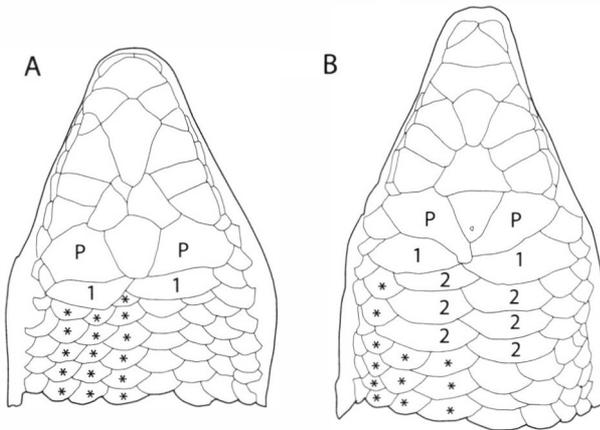


FIG. 4. Details of scales on the nape of two specimens of *Mabuya*: (A) specimen with only a pair of primary nuchals (*M. nigropunctata*, OMNH 36834) and (B) specimen with a pair of primary nuchals and three pairs of secondary nuchals (*M. carvalhoi*, AMNH 13732). P: parietal scale; 1: primary nuchal scale; 2: secondary nuchal scale; *: rows of cycloid scales homologue to nuchals ones (only showed for the left side).

the new species described in the present paper. Until now, no real definition was given to explain what a nuchal scale is, this point being somewhat unclear in the literature. Most of the authors who worked on the systematic of the genus *Mabuya* considered more or less implicitly nuchal scales as any scales present on the nape, in contact with parietals, and larger than other cycloid scales (= small scales covering the body) present on the back, (Dunn, 1936; Hoogmoed, 1973; Avila-Pires, 1995; Rodrigues, 2000). Recently, Mayer & Lazell (2000: 872) proposed that the pair of scales on the nape as a pair of nuchal scales if their combined width is more than 75% the width of the parietals.

However, the width of dorsal scales can vary gradually in some specimens, from the small cycloid scales of the back to the wide scales of the nape. Consequently, it becomes impossible to distinguish the limit between these two categories of scales, the count becoming subjective. Therefore, I propose to present here a more accurate definition for the nuchal scales. This one is based on a hypothesis of homology. A nuchal scale is regarded as an enlarged scale of the nape, occupying transversally the place of two or more rows of dorsal cycloid scale. Thus, the antero-posterior transition from nape to back, between nuchal and dorsal scales is sharp, one longitudinal row of nuchals giving rise to two rows of cycloids. In the case of a gradual variation of width between a single row of small dorsal scale and a single row of large scale on the nape, all those scales will be considered as “enlarged dorsal cycloid ones”, not as nuchals. Additionally, I have distinguished two categories of nuchals (Fig. 4). The “primary pair of enlarged nuchals, or nuchals I” (Fig. 4A-B) is in contact with the posterior border of the parietal scales. Each scale of this always singular pair occupies the equivalent of three transverse rows of dorsal cycloid scales (sometimes two). The nuchals I are present in all species belonging to the genus *Mabuya sensu lato*. The “secondary pairs of

enlarged nuchals, or nuchals II” (Fig. 4B) are aligned along the vertebral axis, posterior to the “nuchals I”. Their number could vary from one to four/five pairs (holotype of *M. berengeriae*) depending on the species. Each “nuchal II” occupies the equivalent of two transverse rows of dorsal cycloid scales. They are always present in six species, *M. berengeriae*, *M. carvalhoi*, *M. croizati*, *M. nigropalmata*, *M. macrorhyncha* and *Mabuya sloanii*. They are nearly always absent in *M. arajara*, *M. bistrata* (*sensu* Avila-Pires, 1995), *M. caissara*, *M. cochabambae*, *M. dorsivittata*, *M. falconensis*, *M. frenata*, *M. guaporicola*, *M. heathi*, *M. pergravis*, *M. mabouya* (*sensu* Miralles, 2005), *M. nigropunctata* (*sensu* Avila-Pires, 1995) and *M. unimarginata* (*sensu* Savage, 2002), (Avila-Pires, 1995; Dunn, 1936; Mausfeld & Lötters, 2001; Mijares-Urrutia & Arends, 1997; Rebouças-Spieker, 1981; Rodrigues, 2000). On the other hand, they seem to be occasionally present in only four species, *M. agmosticha*, *M. lineolata*, *M. macleani* and *M. pergravis* (Cochran, 1941; Dunn & Saxe, 1950; Mayer & Lazell, 2000; Rodrigues, 2000). In conclusion, the characteristic “presence or absence of secondary nuchals scales” constitutes a new useful diagnostic character given its very low intraspecific variability for the majority of *Mabuya* species.

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APPENDIX

SPECIMENS EXAMINED AND LOCALITIES ($n=159$):

Mabuya berengeriae sp. nov. ($n=1$): COLOMBIA: San Andrés Island: UMMZ 127884 (holotype), between Morgans Cave and Punta Sur (= South End).

Mabuya carvalhoi ($n=1$): BRAZIL: Roraima: AMNH R-137372 (paratype), Ilha de Maraca.

Mabuya croizati ($n=14$): VENEZUELA: Sucre: AMNH R-29314 (holotype), Turumiquire massif; CM 7978, 7982, 7988, Elvecia, Turumiquire massif; FMNH 17796, 17797, 17798-428 to 17798-430, 17799-434 to 17799-438, Turumiquire massif.

Mabuya falconensis ($n=5$): VENEZUELA: Carabobo: MHNLS 5511, 5512, Lago de Valencia; FALCÓN STATE: 5526, Sierra San Luis, Curimagua; UMMZ 55927, 55932, Tucacas.

Mabuya mabouya ($n=11$): LESSER ANTILLES: Dominica: MNHN 2003-0838, UMMZ 83323. Guadeloupe: MNHN 2902, 1889-0664. Martinique: MNHN 738, 739, 1785, 5110, 5421, 5424. Unspecified island: MNHN 2903.

Mabuya nigropunctata ($n=98$): BRAZIL: Acre: OMNH 37048 to 37051, 5 Km N Porto Walter, inland from the Rio Juruá. Amazonas: OMNH 37681 to 37698, Município de Castanho, 40 Km S Manaus at Km 12 on road to Autazes. Maranhão: MPEG 10690, 10691, 10693, 10695, 10698, Nova Vida, 25 Km dist. of Rio Gurupi, BR 316. Para: MPEG 8605, 8608, 8611, 8616, 8642, Bela Vista, Viseu; MPEG 12194, 12196, 12199, 12200, 12218 between the rivers Tocantins and Mojú, 12 miles of the barrage Tucuruí; OMNH 36828 to 36842, CEMEX, Agropecuaria Treviso LTDA, 101 Km S and 18 Km E Santarém. Rondonia: OMNH 37411 to 37417, Rio Formoso, Parque Estadual Guajará-Mirim, 90 Km N Nova Mamoré. Roraima: OMNH 36313 to 36322, 7 km E Rio Ajaraní, BR 210. FRENCH GUIANA: MNHN 1996-4570 to 1996-4572, 1997-2206 to 1997-2213, St Eugene; MNHN 2002-0612, 2002-0613, St Marcel. TRINIDAD & TOBAGO: AMNH R-64528, Trinidad island FMNH 49901 to 49908, Trinidad island, San Rafael, UMMZ 79919,

Trinidad island. VENEZUELA: Amazonas: MHNLS 16389, Parima B, Bolívar: MHNLS 15532, Serranía del Supamo, Cerro Santa Rosa; MHNLS 11545, foot of the Roraima Mountain. Delta Amacuro: MHNLS 4543, Burojoida. Sucre: MHNLS 15533, Peninsula de Paria, Las Melenas; MHNLS 16203, Peninsula de Paria, Macuro.

Mabuya lineolata ($n=1$): GREATER ANTILLES: Dominican Republic: AMNH R-42145 (Holotype), Monte Cristy province, not far from Monte Cristy, near the bank of the Rio Yaque del Norte.

Mabuya pergravis ($n=2$): COLOMBIA: Providencia island: USNM 13875 (Holotype), USNM 76947

Mabuya sloanii ($n=19$): BAHAMAS: Turks and Caicos islands: UMMZ117393, West Caicos, on W side. GREATER ANTILLES: Jamaica: UMMZ 85861, Portland Point. Puerto Rico: CM 23775, 23776, FMNH 215, UMMZ 124819, Mona Island; OMNH 32878, unspecified locality. LESSER ANTILLES: Anguilla: CM 115480, Brimegin, W of Fountain Hill, CM 115481, unlocalised locality. British Virgin Islands: CM 17357, 17358, Aneгада; UMMZ 80582 Virgin Gorda. Saint-Barthélemy: MNHN 1997-6064, 2003-0843, 2003-0844, unlocalised locality. US Virgin Islands: MNHN 554 (holotype), 1088, St Thomas; UMMZ 73821, St Thomas, Buck Island; UMMZ 80586, St Thomas, Capella Island.

Mabuya unimarginata complex ($n=7$): COSTA RICA: Puntarenas: UMMZ 117575, 1 Mile E of Volcan de Buenos Aires cone finca; UMMZ 145875, 16 Km S San Vito, on Highway 16 Las Cruces field station, 1250m. MEXICO: Yucatan: AMNH R-38863 to R-38865, Chichen Itza; UMMZ 113548, Calcehtok. NICARAGUA: Leon: OMNH 36173 to 36175, Vulcan Momotombo. Rio San Juan: OMNH 36177 to 36179, by Isla de Diamante on Rio San Juan. Rivas: OMNH 36176, Chococente. PANAMA: Bocas del Toro: UMMZ 142636, Bocas del Toro. Los Santos: CM 43593 to 43594, Los Santos; CM 43595, Santa Maria. Panama: UMMZ 135354, Gorgas Memorial Laboratory Field Station on the Rio Bayano, 250 m alt.

SEXUAL DIMORPHISM IN TWO SPECIES OF EUROPEAN PLETHODONTID SALAMANDERS, GENUS *SPELEOMANTES*

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Speleomantes ambrosii and *S. strinatii* are morphologically similar but genetically well differentiated plethodontids inhabiting north-western Italy. Ten morphological characters were used to assess the amount of intraspecific sexual dimorphism in both species. On average adult females were 10% and 7% longer than males in *S. ambrosii* and *S. strinatii* respectively. ANCOVA showed that in both species there were no differences in body proportions between males and females of equal size. Multivariate analyses of size-adjusted morphological characters showed that species differed significantly in body shape, while sexes within species did not show significant overall body shape differences. The observed pattern of sexual size and shape dimorphism was similar in both species of *Speleomantes* and is discussed in relation to the reproductive biology of plethodontids.

Key words: *Hydromantes*, morphometrics, terrestrial salamanders

INTRODUCTION

In salamanders sexual size dimorphism (SSD) is usually not great, with females growing slightly larger than males (Duellman & Trueb, 1986). However, in the salamander family Plethodontidae different patterns of SSD are observed (Bruce, 2000). In following the traditional taxonomy of Wake (1966), in the subfamily Desmognathinae males outgrow females in most species (Bruce, 1993; Bakkegard & Guyer, 2004), while in the tribes Hemidactyliini, Plethodontini and Bolitoglossini, all belonging to the subfamily Plethodontinae, SSD, when expressed, tends to be female-biased (Shine, 1979; Bruce, 2000). The European plethodontid genus *Speleomantes* Dubois 1984, traditionally assigned to the tribe Bolitoglossini, is no exception, as in all species females reach a larger maximum size than males (Lanza *et al.*, 1995; Lanza, 1999). However, in the North American *Hydromantes*, the sister genus to *Speleomantes*, at least one species, *H. platycephalus*, shows male-biased SSD (Adams, 1942). Moreover, recent molecular studies have shown that *Speleomantes* is allied to the plethodontine genus *Aneides* (Mueller *et al.*, 2004), wherein the pattern of SSD is variable (Staub, 1989).

To date seven species of *Speleomantes* have been described, three from south-eastern France and continental Italy and four from Sardinia, (Nascetti *et al.*, 1996; Lanza, 1999). Life histories of *Speleomantes* are characterized by complete terrestriality, egg brooding by females (Durand, 1967), and relatively long generation times. North-western Italian *S. strinatii* males become mature in their third year and females one year later [Salvidio, 1993 (at that time *S. strinatii* was considered conspecific with *S. ambrosii*)].

In *Speleomantes* the main sexually dimorphic characters are the presence in reproductive males of a well-developed chin gland, a swollen vent, and monocuspid premaxillary teeth (Greven *et al.*, 2004). According to Lanza *et al.* (1995) sexes differ in that females grow larger and males possess more projecting snouts and longer limbs. However, in their morphological analyses, Lanza *et al.* (1995) treated the two north-western Italian species, *S. ambrosii* and *S. strinatii*, together, concealing possible morphological differences between these genetically well separated taxa (Nascetti *et al.*, 1996). In addition, sexually dimorphic characters were analysed using body ratios to compare populations and species but the amount of intraspecific sexual dimorphism was not evaluated (Lanza *et al.*, 1995). Thus, the principal aims of this paper were (1) to assess the presence and amount of sexual size and shape dimorphism within each species, and (2) to determine whether any observed pattern in sexual shape dimorphism showed similar trends in the two species. We also deemed it useful to examine trends in SSD in these species with reference to the principles of sexual selection and life history theory, relative to variation in SSD, life history, and reproductive behaviour in bolitoglossine and other plethodontid salamanders.

MATERIAL AND METHODS

STUDY SPECIES AND SAMPLED POPULATIONS

Speleomantes ambrosii and *S. strinatii* are large bolitoglossines with maximal total lengths of about 125 mm. The two species are similar in external morphology but genetically well differentiated, in that Nei's mean genetic distance, calculated over 33 genetic loci by starch gel electrophoresis, was $D=0.33$, range 0.26-0.44, (Nascetti *et al.*, 1996). In addition, no genetic introgression was observed between two parapatric

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populations separated by a linear distance of only 1 km (Nascetti *et al.*, 1996).

In this study, 80 salamanders were measured: 40 *S. strinatii* (20 male, 20 female) collected inside two artificial cavities near Savignone (Genova), central Liguria, between 25 July and 1 September 2003, and 40 *S. ambrosii* (20 male, 20 female) captured on 5 August 2003 inside an artificial cavity near the town of La Spezia, eastern Liguria. All measurements were taken on live sexually mature salamanders: males possessing a chin gland and females measuring more than 58 mm from the snout to posterior margin of the cloaca (Salvidio, 1993; Salvidio *et al.*, 2003). After being measured all salamanders were released at capture sites.

MORPHOMETRIC MEASUREMENTS

Body dimensions were measured in living animals. Snout-vent length from the tip of the snout to the posterior edge of the cloaca (SVL_p) and snout-vent length from the tip of the snout to the anterior edge of the cloaca (SVL_a) were measured with a transparent plastic ruler to the nearest mm. Cloacal length was obtained by subtracting SVL_a from SVL_p . The following measurements were taken with vernier calipers to the nearest 0.1 mm: head length from the tip of the snout to the jaw articulation; head width as the distance between right and left jaw articulations; head depth at the level of the jaw articulations; internarial distance; orbitonarial distance from the anterior eyelid commissure to the naris; interorbital distance between anterior eyelid commissures at the proximal edge of the canthus rostralis; eye diameter; anterior body depth at the level of forelimb insertions; posterior body depth at the level of hindlimb insertions; anterior body width just anterior to the insertion of forelimbs; posterior body width just anterior to the hindlimb insertions; forearm length from the elbow to the wrist; thigh length from the leg insertion to the knee; shank length from the knee to the ankle; diameter of the brachium midway; and thigh diameter midway. Bilateral characters were measured on the right side.

To estimate measurement error, five male *S. strinatii* were selected at random and measured 10 times for all characters. Values of the coefficient of variation ($CV = SD/mean$) for each character were calculated for each individual, and, following Hayek *et al.* (2001), only characters showing the highest repeatability (i.e., those displaying a mean $CV < 0.05$) were selected for further analyses.

STATISTICAL ANALYSES

A Mann-Whitney non-parametric test was used to compare SVL_a between sexes, and analysis of covariance (ANCOVA) was used to assess differences in log-transformed variables between sexes using log- SVL_a as covariate. As all transformed variables were highly correlated with body length ($P < 0.005$ in all cases), the overall morphological variation could simply reflect body size variation. Different methods have been

proposed to obtain size-adjusted morphometric variables (see references in Adams & Beachy, 2001; Parsons *et al.*, 2003). In this paper size-adjusted variables were obtained using residuals from the regression of log-transformed variables against log- SVL_a (e. g., Miles, 1994; Carr, 1996; Adams, 2000). Residuals were used in principal component analysis (PCA) and in discriminant function analysis (DFA) with cross validation. The generalised Mahalanobis distance (D^2) was used to estimate the dissimilarity between groups (Carr, 1996; Adams, 2000). Pairwise post-hoc comparisons were performed by means of F -ratios using Hotelling's statistics calculated from Mahalanobis distances (Manly, 2000). As group means were tested six times, the level of significance for rejecting the null hypothesis of no difference was set to 0.008, using Bonferroni adjustment.

Although not formally presented, similar statistical conclusions were obtained using the set of scores on PC2-PC6 of transformed variables excluding those on the first principal component (PC1) that is often considered a general size factor (Somers, 1986; Adams, 2000; Adams & Beachy, 2001). In the present study, the PC1 using the untransformed variable set accounted for 54% of the total variance.

The degree of sexual dimorphism was evaluated by dividing the larger sex mean size by the smaller with the result being arbitrarily positive when females are larger and negative in the opposite case (Gibbons & Lovich, 1990). All statistics were performed using MINITAB 12.21 release.

TABLE 1. Mean coefficients of variation (CV) and ranges for morphological characters in five randomly selected male *S. strinatii*, each measured 10 times.

Measurement (mm)	Mean CV	Range	Selected for analysis
SVL_p	0.01	0.01-0.02	
SVL_a	0.02	0.01-0.02	covariate
Cloaca	0.10	0.05-0.14	
Head length	0.03	0.01-0.04	yes
Head width	0.03	0.03-0.04	yes
Head height	0.04	0.03-0.07	yes
Internarial distance	0.08	0.07-0.10	
Interorbital distance	0.05	0.04-0.06	yes
Eye length	0.07	0.06-0.08	
Orbitonarial distance	0.09	0.06-0.10	
Ant. body height	0.07	0.03-0.10	
Post. body height	0.06	0.04-0.08	
Ant. body width	0.05	0.03-0.06	yes
Post. body width	0.07	0.04-0.08	
Arm length	0.03	0.02-0.04	yes
Thigh length	0.09	0.06-0.12	
Shank length	0.04	0.03-0.04	yes
Arm diameter	0.05	0.04-0.06	yes
Thigh diameter	0.05	0.04-0.06	yes

TABLE 2. Means and standard deviations for morphological characters measured in *S. ambrosii* and *S. strinatii*. ANCOVA comparing sexes within species was based upon log-transformed selected characters using log-SVL_a as a covariate.

Measurement (mm)	<i>Speleomantes ambrosii</i>			<i>Speleomantes strinatii</i>		
	Males (n=20)	Females (n=20)	ANCOVA P	Males (n=20)	Females (n=20)	ANCOVA P
SVL _p	60.10±2.61	66.35±3.27		60.50±3.05	64.50±4.22	
SVL _a	54.45±2.20	59.80±3.04		54.45±2.96	58.05±3.63	
Cloaca	5.65±0.88	6.55±0.95		6.05±1.91	6.45±1.05	
Head length	9.69±0.76	10.08±0.92	0.372	9.12±0.52	9.74±0.97	0.669
Head width	8.67±0.40	9.47±0.63	0.465	8.34±0.62	8.73±0.71	0.260
Head height	4.33±0.23	4.73±0.40	0.808	4.26±0.23	4.49±0.32	0.234
Internarial distance	3.30±0.21	3.40±0.26		3.56±0.31	3.46±0.41	
Interorbital distance	4.51±0.40	4.72±0.39	0.185	4.39±0.37	4.53±0.40	0.080
Eye length	3.51±0.24	3.74±0.32		3.23±0.29	3.38±0.34	
Orbitonarial distance	3.19±0.24	3.22±0.31		3.14±0.20	3.04±0.34	
Ant. body height	5.85±0.48	6.43±0.51		6.03±0.43	6.14±0.58	
Post. body height	5.61±0.28	5.84±0.52		5.83±0.37	5.82±0.60	
Ant. body width	7.42±0.75	8.30±0.73	0.162	7.90±0.70	7.22±0.46	0.080
Post. body width	6.14±0.66	6.79±0.47		6.38±0.54	6.69±0.51	
Arm length	6.71±0.33	7.04±0.38	0.601	6.71±0.39	6.82±0.51	0.424
Thigh length	5.88±0.46	5.99±0.53		6.05±0.58	6.19±0.62	
Shank length	6.48±0.35	6.93±0.44	0.510	6.67±0.41	6.87±0.46	0.210
Arm diameter	1.59±0.11	1.78±0.18	0.739	1.72±0.27	1.88±0.20	0.420
Thigh diameter	1.89±0.12	2.03±0.16	0.920	1.97±0.22	2.19±0.23	0.189

RESULTS

Means and standard deviations of SVL_a were 59.80±3.04 (range 53-66) in females and 54.45±2.20 in males (range 51-60) in *S. ambrosii*, and 58.05±3.63 in females (range 52-64) and 54.45±2.96 in males (range 49-59) in *S. strinatii*. In both species females were significantly larger than males ($W=571.5$, $P<0.001$ and $W=514.5$, $P=0.005$, for *S. ambrosii* and *S. strinatii* respectively). The values of the SSD index were +1.10 for *S. ambrosii* and +1.07 for *S. strinatii*.

Mean CV values of morphometric measures are shown in Table 1. Overall, CVs ranged from 0.01 (SVL_p) to 0.10 (cloaca length) and were normally distributed (Kolmogorov-Smirnov one-sample test $P>0.15$). Apart from SVL, morphometric characters based on rigid landmarks (e.g. head bones and limbs) showed the lowest CV values; on the other hand those based on fleshy structures (e.g. nostrils and eyes) displayed moderate to high variability. In both species there were no intersexual differences in the nine characters selected for their low measurement variability (ANCOVA, $P>0.07$ in all cases, Table 2).

The result of a PCA using nine size-adjusted variables is shown in Fig. 1. The scatterplot obtained plotting PC1 and PC2 explained 42% of the total variance and showed a certain degree of shape differentiation between species, which were partially separated on PC2. The variables having the highest contribution to PC2 were: arm diameter (0.569), head length (-0.347), forearm length (-0.373) and head width (-0.293).

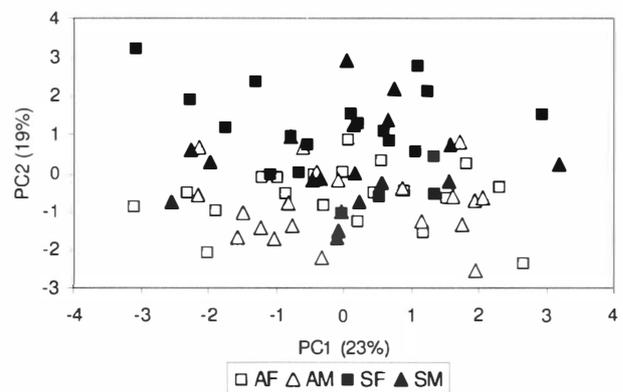


FIG. 1. Scatterplot of PC1 versus PC2 explaining 42% of the total variance. AF = *S. ambrosii* females, AM = *S. ambrosii* males, SF = *S. strinatii* females, SM = *S. strinatii* males.

On the other hand, sexes within species widely overlapped. A MANOVA using the scores of the first four PC axes (with eigenvalues > 1.0 and explaining 67% of the total variance), with sex and species as models, showed a highly significant differentiation between species (Wilks' lambda = 0.597, $F=12.331$, $df=4,73$, $P<0.001$), while sexes and the interaction between species and sex were nonsignificant (Wilks' lambda = 0.911, $F=1.764$, $df=4,73$, $P=0.145$, and Wilks' lambda = 0.949, $F=0.984$, $df=4,73$, $P=0.422$, for sexes and interaction respectively). Discriminant function analysis with cross validation correctly classified to species 78% of individuals (62 out of 80), and 41% (33 out of 80) were correctly classified to the corresponding species and sex (Table 3). The Mahalanobis distances indicated

TABLE 3. Results of a discriminant function analysis with cross validation based on nine size-adjusted morphological characters in two species of *Speleomantes* from north-western Italy.

Classified in group	True group			
	<i>S. ambrosii</i> female	<i>S. ambrosii</i> male	<i>S. strinatii</i> female	<i>S. strinatii</i> male
<i>S. ambrosii</i> female	10	7	2	3
<i>S. ambrosii</i> male	7	10	3	4
<i>S. strinatii</i> female	1	1	7	7
<i>S. strinatii</i> male	2	2	8	6
Total <i>N</i>	20	20	20	20
<i>N</i> correct (proportion)	10 (0.50)	10 (0.50)	7 (0.35)	6 (0.30)

that the amount of shape differentiation between sexes within species was similar ($D^2=1.29$, $D^2=0.92$) and did not differ significantly ($F=1.135$, $df=9,30$, $P>0.008$, and $F=0.810$, $df=9,30$, $P>0.008$, for *S. strinatii* and *S. ambrosii* respectively). However, the two species appeared relatively well-separated ($2.65 < D^2 < 5.59$). The between-species D^2 values differed significantly (F -ratios > 3.5 , $df=9,30$; $P<0.008$) with only one exception (*S. ambrosii* females - *S. strinatii* males).

DISCUSSION

We found that *S. ambrosii* and *S. strinatii* differed statistically in overall shape, especially in the relative dimensions of head and forelimbs. In both species adult females outgrow males by 10% (*S. ambrosii*) and 7% (*S. strinatii*), confirming earlier studies of female-biased SSD in *Speleomantes* (Lanza *et al.*, 1995). The female-biased pattern of SSD conforms to that seen in most bolitoglossines and plethodontines (Bruce, 2000), exceptions being *Thorius magnipes* (SSD index = -1.06; from Table 4 in Bruce, 2000), *Hydromantes platycephalus* (SSD index = -1.04; from Table 1 in Adams, 1942), and several species of *Aneides* (Staub, 1989). Given the sister group relationship of (1) *Speleomantes* and *Hydromantes*, and (2) *Speleomantes-Hydromantes* and *Aneides*, as proposed recently by Mueller *et al.* (2004), the variation in SSD in these taxa deserves further study. The values of SSD observed in *Speleomantes* in the present study fell in the lower range of those calculated from Bruce (2000: Tables 3 and 4) for the Bolitoglossini, in which the SSD index was on average 12% (value calculated from 27 species with both sexes represented by at least 10 individuals, including *T. magnipes*). No differences between sexes in single morphological characters were observed and multivariate shape dimorphism was slight in both *Speleomantes* species, indicating that males and females had similar overall body proportions. These results were different from those obtained in aquatic salamanders, such as *Triturus* and *Euproctus*. In the former genus, males differ from females in having longer front and hind limbs (Dandová *et al.*, 1998; Malmgren & Tholleson, 1999), while in the latter males possess wider and longer heads, stouter bodies, and longer limbs (Serra-Cobo *et al.*, 2000). In both cases, the observed shape dimorphism may be directly related to the mating

system, since in large *Triturus* species males establish and defend small territories in which they display themselves to attract females (Malmgren & Tholleson, 1999; Pough *et al.*, 2001), whereas in *Euproctus* the male engages in direct physical contact with the female, grasping her with his jaws and pushing a spermatophore toward her vent with his hindlimbs (Duellman & Trueb, 1986).

Larger size at first reproduction in females versus males is apparently a reflection of greater age at first reproduction in the former sex, estimated as 5 yr in females versus 3.5 yr in males in *S. strinatii* (Salvidio, 1993). Such a difference carries over to greater average and maximum sizes in females, as shown in *S. strinatii* by Salvidio (1993) and in both *S. strinatii* and *S. ambrosii* in the present study.

A suite of reproductive traits of female plethodontids appears to regulate adult body size. First, in female salamanders generally (Salthe, 1969), and in plethodontids specifically (Tilley, 1968; Bruce, 1969), including bolitoglossines (Houck, 1977*a,b*), clutch size and overall clutch dimensions tend to increase with body size, although such a relationship has not been investigated in *Speleomantes*. Secondly, growth tends to decline following maturation in female plethodontids (Marvin, 2001). And third, in most plethodontids, including some species of *Speleomantes* (Durand, 1967; Mutz, 1998), the female parent attends the egg clutch until hatching (Crump, 1995; Nussbaum, 2003). This set of traits may reflect the operation of fecundity selection, wherein larger females gain a selective advantage because they produce larger clutches and can better protect developing clutches from predators, pathogens, and desiccation. However, if growth to larger size requires a longer pre-reproductive developmental period, then, other factors being equal, growth and development to larger body size must entail a fitness cost incurred by mortality in the period of delay. Thus, trade-offs among these factors are expected to yield an optimal age/size at first reproduction as an outcome of selection in females. Alternatively, if larger size is attained by increase in growth rate, without effect on age at first reproduction, the resulting gains in fecundity may be offset by increased mortality from reductions in the allocations for repair, maintenance, and defense (Arendt, 1997). Limited data suggest that the former pathway is more

important in plethodontids (Marvin, 2001; Bruce, 2003), but data for *Speleomantes* are lacking.

If female reproductive success can be measured by fecundity and the efficacy of parental care, the comparable trait in males is success in inseminating females (Roff, 2002). In plethodontids, where courtship involves a complex tail-straddling walk, fixed at the family level (Houck & Arnold, 2003), selection for male size at first reproduction may be determined as the minimum or threshold size at which a male can successfully mate. In *Speleomantes strinatii* and *S. ambrosii*, as in most bolitoglossines, size at first reproduction in males is slightly less than that of females and this difference is achieved by a lower age at first reproduction in males.

Life-history theory predicts that smaller adult size in males versus females may be an outcome of selection in species in which competition among males for mates involves either a scramble, as in some explosive breeders, or mate searching, with minimal direct contact between males (Pough *et al.*, 2001). In contrast, selection for larger male size may occur under conditions of contest competition, involving male-male aggression and/or territorial defence of mating sites (Andersson, 1994; Shuster & Wade, 2003). In desmognathine plethodontids, where male-biased SSD is the rule in most species (Bruce, 1993, 2000; Bakkegard & Guyer, 2004), aggressive interactions have been observed between males during courtship, reflecting contest-mate competition (Houck, 1988). In *Speleomantes*, where mating seasons seem prolonged and sex ratios close to 1:1 (Salvidio, 1993; Salvidio *et al.*, 2003), there is no evidence of male aggressiveness (Salvidio, personal observation). Thus we hypothesise that female-biased SSD in *Speleomantes* (and perhaps in other bolitoglossines) may correlate with an exploitative, mate-searching mating system. Experimental studies are needed to test this hypothesis.

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SEASONAL AND HOURLY PATTERNS OF REPRODUCTIVE ACTIVITY IN *SCINAX TRAPICHEIROI* (ANURA, HYLIDAE), RIO DE JANEIRO STATE, SOUTH-EASTERN BRAZIL

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We examine the temporal pattern of reproduction of the hylid *Scinax trapicheiroi* and evaluate how environmental factors affect the calling activity of males and the reproductive activity of females. Fieldwork was carried out at a small stream inside the Atlantic Rainforest of Ilha Grande, an island off the coast of south-eastern Brazil. The study area was sampled monthly from October 2000 to September 2001. Periods for quantifying males' calling activity were alternated with periods of observing female presence. Calling activity occurred mainly during the night. Males called during all months of the year, but activity was higher during the warm wet season. The number of calling males positively influenced the number of calls emitted per male. Fewer females appeared at the stream to reproduce than males. The presence of females was affected only by rainfall during the previous day.

Key words: Atlantic Rainforest, behaviour, calling activity, environmental factors, frog

INTRODUCTION

In anuran species with long reproductive seasons, the reproductive success of males typically depends on their ability to attract females to their calling site because female arrival is unpredictable (Wells, 1977). Knowing the temporal patterns of vocalization of anuran species (e.g. continuous, opportunistic, explosive) helps us to understand how variations in environmental factors (such as temperature, humidity or rainfall) might affect their reproductive activity. Several studies have shown that calling activity of anurans is influenced by local environmental factors, including relative humidity (e.g. Cree, 1989), air temperature (e.g. Pough *et al.*, 1983; Navas, 1996; Boquimpani-Freitas *et al.*, 2002), light intensity (Hatano *et al.*, 2002), photoperiod (e.g. Hatano *et al.*, 2002), rainfall (e.g. Donnelly & Guyer, 1994), or a combination of environmental factors (Oseen & Wassersug, 2002; Murphy, 2003). However, the relative importance of local and microscale variation in physical and environmental conditions on calling activity of males may differ among species (Brooke *et al.*, 2000).

In this study we investigate calling and reproductive activity of *Scinax trapicheiroi*. The genus *Scinax* is presently divided into five groups of species, the *S. rubra*, *S. rostrata*, *S. catharinae*, *S. staufferi* and *S. perpusilla* groups (Faivovich, 2002). *Scinax trapicheiroi* (B. Lutz, 1954) is a small hylid frog from the *S. catharinae* group which inhabits mountainous areas in the Atlantic Rainforest region of southeastern Brazil (Carvalho-e-Silva & Carvalho-e-Silva, 1994; Faivovich, 2002). In these areas, *S. trapicheiroi* repro-

duces in clear waters of small and shallow rivulets (either standing or slow moving water) or temporary ponds (Rico *et al.*, 2004). Studies on *S. trapicheiroi* are limited to the species and tadpole description, taxonomy and geographic distribution (Lutz, 1954, 1973; Carvalho-e-Silva & Carvalho-e-Silva, 1994). Information on ecological aspects of *S. trapicheiroi* is restricted to its breeding biology (Rico *et al.*, 2004). Our specific aim was to address the effects of air temperature, relative humidity, photoperiod and rainfall on hourly and monthly reproductive activity of both males and females in our study area.

MATERIAL AND METHODS

The study was carried out from October 2000 to September 2001 at a small stream in the Atlantic Rainforest of Ilha Grande (23°12'S, 44°13'W). Ilha Grande is located in southern State of Rio de Janeiro and is one of the largest islands on the Brazilian coast. The landscape of the island is rugged, and the highest peaks reach 1000 m. The island is covered by Atlantic Rainforest with different levels of regeneration due to disturbance by human activities (Araújo & Oliveira, 1988). Our study site was a creek whose origin was a steep forested hillside. Water levels in the creek were highly variable, and during periods of no rainfall the stream was reduced to a linear series of disjunct pools fed by subsurface flow.

Four sampling points (separated by at least 30 m) were established along 150 m of the creek. Male calling activity was quantified monthly at each point, during 5 min/point/hr over a 24-hr sampling regime. We included all males calling from within an imaginary circle with 10 m radius and tallied calls by hand. To reduce the probability of pseudoreplication (Hulbert, 1984), we recorded calling activity in eight, 3-hr sessions, so as to cover the 24 hrs of a day, in each of these four points. These 3-hr sessions were alternated during one week of

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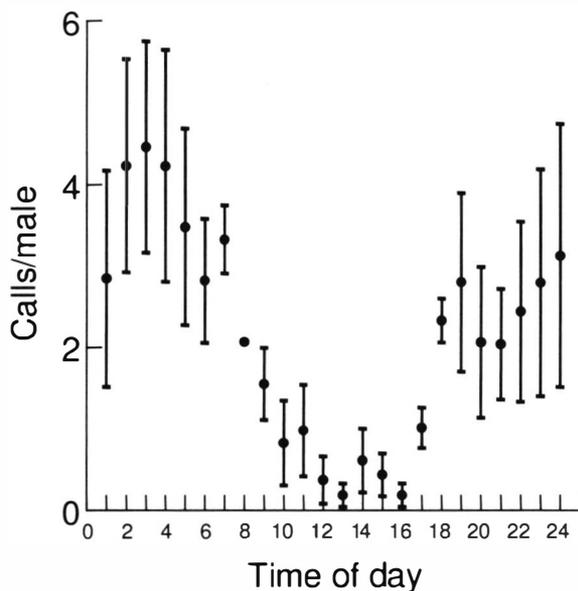
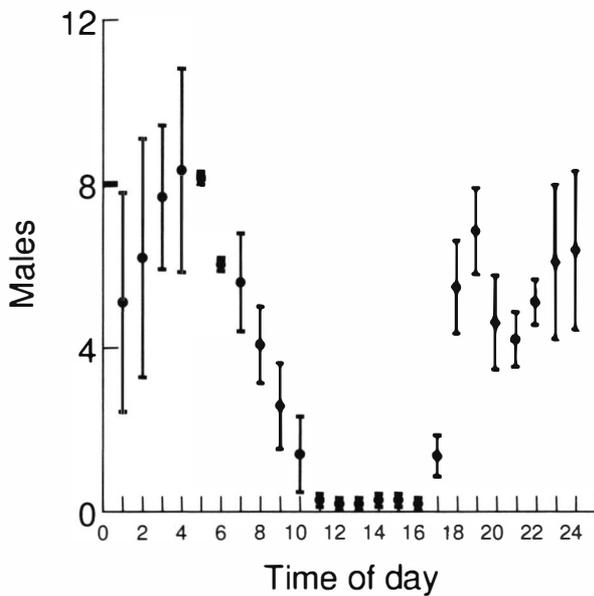


FIG. 1. Hourly pattern of calling activity of males *Scinax trapicheiroi* along a stream in Ilha Grande. Figures represent the mean numbers (± 1 SD) of males calling (a), and calls/male (b), during 5 minutes/hour per point, from October 2000 to September 2001.

fieldwork (for example, on day 1 we sampled the periods 0100-0400 hrs; 0900-1200 hrs; and 1500-1800 hrs; on day 2 we sampled the periods 0400-0700 hrs; 1200-1500 hrs, and so on). Calling activity was expressed as the total number of males calling and as the mean number of calls per male (Heyer *et al.*, 1994).

Female annual activity was estimated based on their presence at the study area, on three nights each month (see Rico *et al.*, 2004 for details). These nights ($n=36$) were not the same as those of the calling activity samplings and were not consecutive. Because we did not recapture any female, even within a month, we assume that each sampling night was independent of the others. The number of females observed and the number of

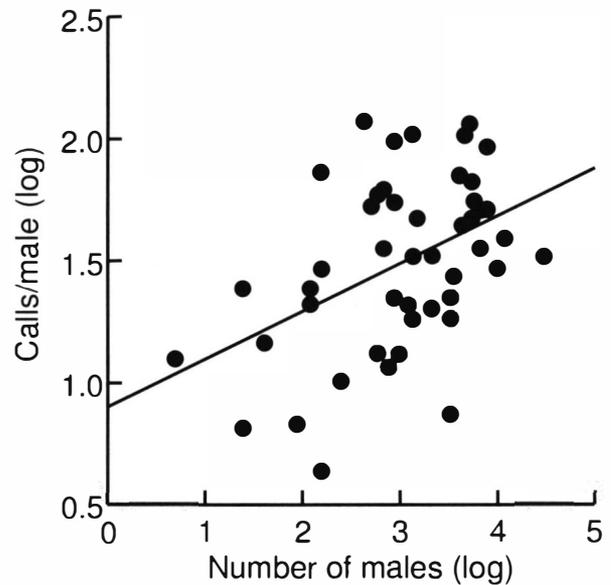


Fig. 2. Number of calls emitted per male in relation to the number of males of *S. trapicheiroi* calling in the chorus along a stream in Ilha Grande.

TABLE 1. Partial regression values (coefficients and significance) of the environmental variables of photoperiod and rain of the previous day for the mean number of males of *Scinax trapicheiroi*, and mean number of calls/male along the year along a stream of Ilha Grande.

	Photoperiod	Rain during previous day
No. of males	0.055, $P=0.716$	-0.010, $P=0.949$
No. of calls/male	0.661, $P<0.001$	0.186, $P=0.086$

clutches deposited in the stream during those nights were used as an index of their reproductive activity.

Air temperature and relative humidity were recorded every hour, and rainfall and photoperiod were recorded daily. Daily rainfall data were obtained from a tipping bucket pluviometer (Davies Rain™) that was placed approximately 1 km from the study area. For our analyses, we used amount of rainfall of the day before each sampling.

To test the effect of the environmental variables on the monthly calling activity of males and reproductive activity of females of *S. trapicheiroi* we used multiple linear regression (Zar, 1999). Male monthly calling activity was related to photoperiod and rainfall considering each sampling point separately ($n=48$), and female activity (number of females present at the creek each sampling night) was related to air temperature and rainfall. We tested whether the hourly activity of calling males (mean of all months and sampling point) differed along the 24-hr period using Repeated Measures ANOVA (Zar, 1999). We assessed the effect of chorus size (log-transformed number of males) on calling intensity (log-transformed number of calls emitted per male) using linear regression. We considered each sampling point separately.

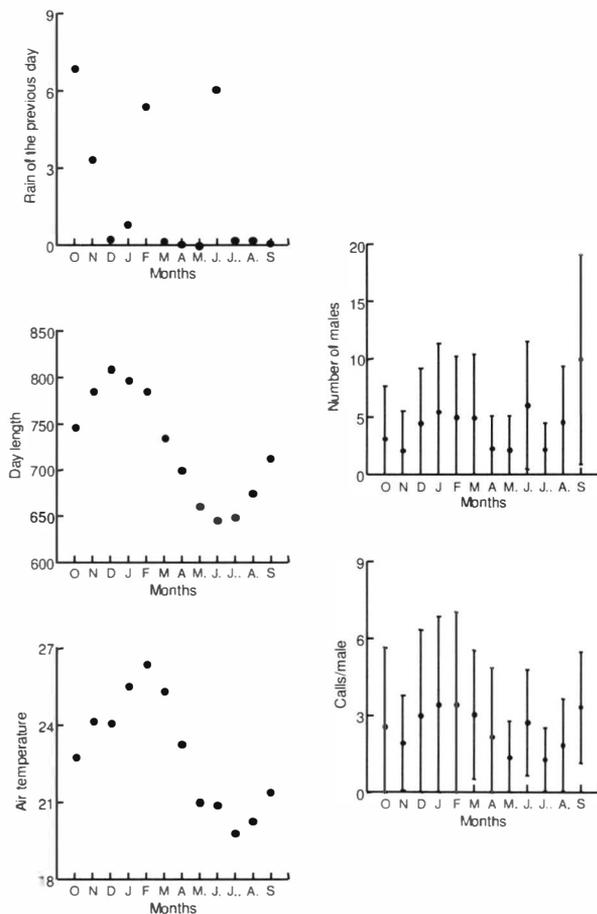


FIG. 3. Annual pattern of rainfall (of the previous day, in mm – upper left), photoperiod (in seconds – middle left), air temperature (in °C – lower left) and mean numbers (± 1 SD) of male *Scinax trapicheiroi* calling (upper right), and calls/male (lower right), during 5 min/point/hr, at four points along a stream in Ilha Grande from October 2000 to September 2001.

RESULTS

HOURLY ACTIVITY

Males of *Scinax trapicheiroi* called mainly during the night, with sporadic vocalizations during the day (Fig. 1). Calling began at sunset (1700-1800 hrs) and quickly increases in activity. In the following three hours, calling activity decreased slightly. Peak activity occurred between 0200 and 0500 hrs, which was followed by a steady decrease after sunrise (0500-0600 hrs). Calling activity significantly differed along the day (Repeated Measures ANOVA: number of males; $F_{23,253}=9.19$, $P<0.001$; mean number of calls per male; $F_{23,253}=11.09$, $P<0.001$)

The number of calls per male increased as the number of males calling in the chorus increased ($R^2=0.22$, $F_{1,46}=13.18$, $P=0.001$; Fig. 2). When more than 15-20 males were calling in the chorus, the number of calls per male no longer increased, but male aggregations usually had fewer than 20 individuals.

MONTHLY ACTIVITY

Calling activity of *S. trapicheiroi* varied during the year (Fig. 3). The highest number of males calling oc-

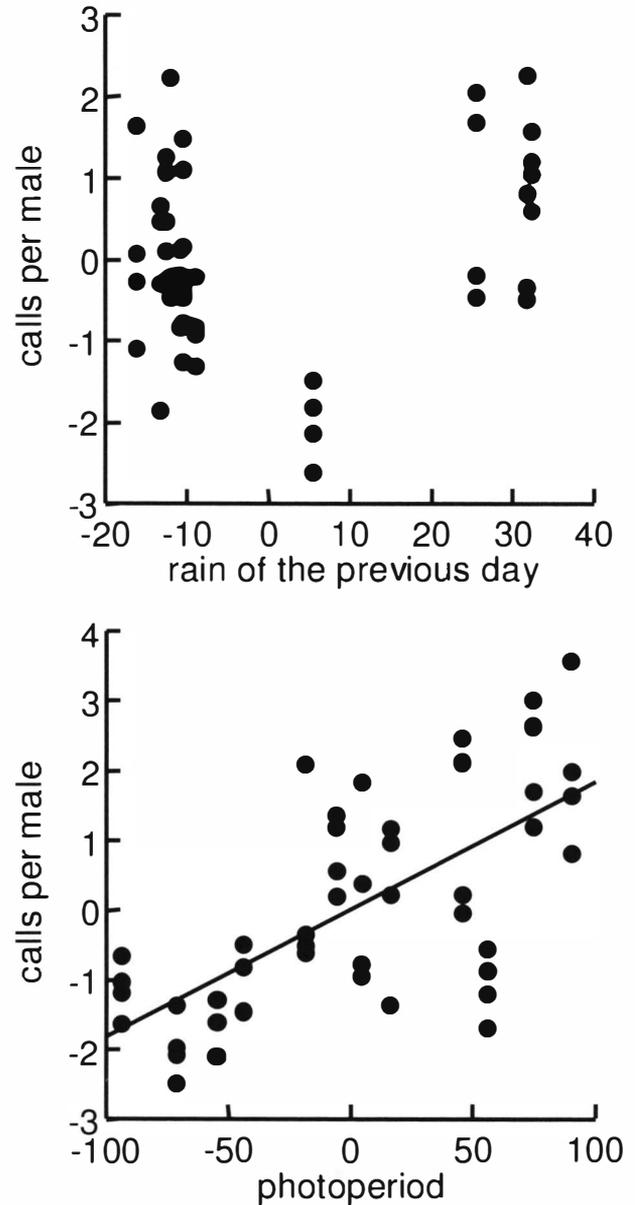


FIG. 4. Partial regression plots depicting the relationship between two environmental variables (rain of the previous day and photoperiod) and the monthly number of calls/male of *Scinax trapicheiroi* at four points along a stream in Ilha Grande.

curred in September 2001, but the mean numbers of calls/male were usually higher from December to February. The number of males calling was not affected by the environmental variables tested (photoperiod and rainfall; $R^2=0.003$; $F_{2,45}=0.067$, $P=0.935$) but the intensity of male calling activity was affected by these variables ($R^2=0.509$, $F_{2,45}=23.28$, $P<0.001$; Table 1). Photoperiod explained ($P<0.001$) an additional part of the variation in number of calls emitted per male (Fig. 4).

FEMALE ACTIVITY

Females were observed in 20 out of 36 sampling nights (55.6%) during the study. The number of new clutches laid per night was highly correlated to the number of females observed that night at the rivulet

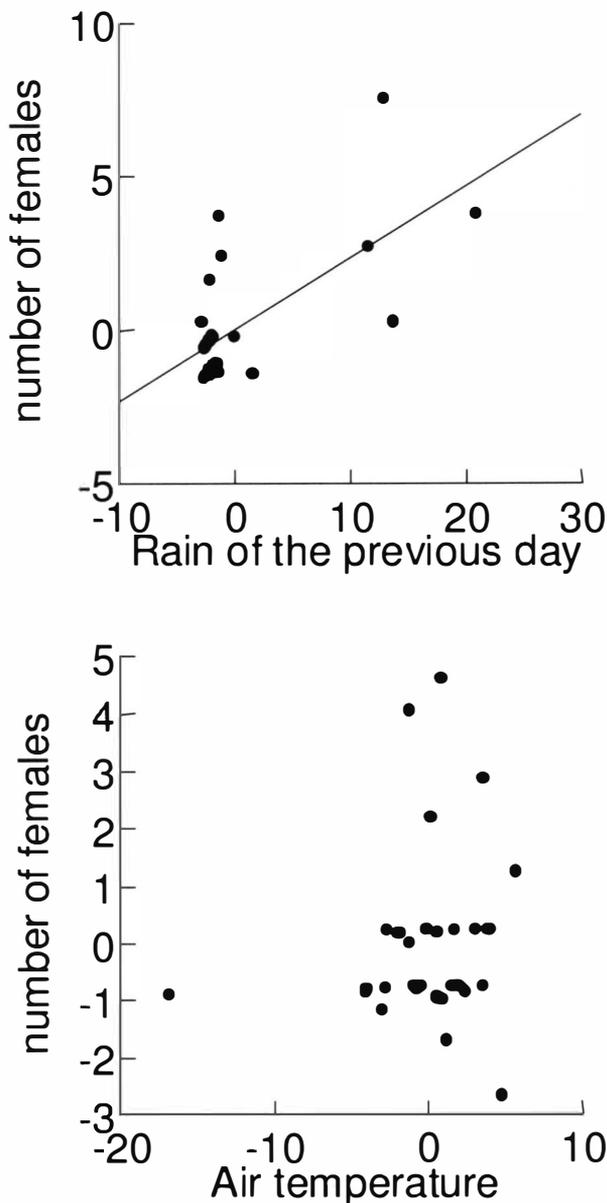


FIG. 5. Partial regression plots depicting the relationship between two environmental variables (air temperature and relative humidity) and the number of females of *Scinax trapicheiroi* along a stream in Ilha Grande, during three nights each month from October 2000 to September 2001.

($R^2=0.13$, $F_{1,34}=5.24$, $P<0.001$). The number of females present at the rivulet was significantly affected by the environmental variables (rain of the previous day and air temperature; $R^2=0.45$, $F_{2,30}=12.20$, $P<0.01$). Only the rain of the previous day (partial coefficient = 0.66; $P<0.001$) explained an additional part of female activity (Fig. 5).

DISCUSSION

There was considerable variation in the number of males *Scinax trapicheiroi* calling and in the number of calls per male, both on a seasonal and on a diurnal scale, which is similar to results of other studies of anuran calling activity (Brooke *et al.*, 2000 and references therein, Murphy 2003). Males of *S. trapicheiroi* called almost

exclusively during the night, as is true for most treefrog species (Duellman, 1989). Sunset and sunrise seem to play an important role in the regulation of the start and of the end of the calling activity. During days with high reproductive activity (inferred from the number of females and of clutches laid at the rivulet), males also called during the first sunlight hours, a behaviour similar to that of other opportunistic hylid species (Bastos, 1996; Pombal, 1997). Differences in hourly activity trends among anuran species can result from differences in foraging schedules (Cardoso & Haddad, 1992), differences in strategies used to avoid predation, dehydration and high temperatures (Cardoso & Haddad, 1992; Pombal, 1997), division of acoustic space (Pombal, 1997; Garcia-Rutledge & Narins, 2001), or phylogenetic history (Bridges & Dorcas, 2000).

Males called every month during the study, but calling intensity varied during the year, being some what higher during months of the warmer wet season (December to February). September 2001 was the exception due to the exceptionally high number of males (more than 20) calling in the chorus in five sampling periods. Our results suggest that there is no direct effect of environmental factors on the number of active males of *S. trapicheiroi*, but rain and photoperiod affected the intensity of calling (expressed as the number of calls emitted per male). We can suppose that a frog species that maintains calling and reproductive activity throughout the year should be less susceptible to changes in environmental factors. Brooke *et al.* (2000) found differences in the effect of large-scale weather factors and small spatial ones. Seasonality in calling activity has also been pointed out for other anuran species (Pough *et al.*, 1983; Hatano *et al.*, 2002) that take advantage of seasonal environmental conditions (temperature, humidity, pluviosity, etc.) to facilitate their calling and reproductive activity. The impact of photoperiod, air temperature and relative humidity on the calling activity of anurans is well known (Whittier & Crews, 1987; Cree, 1989), and also in sympatric anurans at Ilha Grande (Boquimpani-Freitas *et al.*, 2002; Hatano *et al.*, 2002).

Rainfall is the main environmental variable influencing the calling activity of many anurans in tropical and subtropical regions (Aichinger, 1987; Donnelly & Guyer, 1994), due to increasing humidity and the number of water bodies in the habitat. In the study area, short-term rainfall positively influenced the presence of females at the creek, probably as a consequence of the presence of adequate oviposition sites in the habitat. However, heavy rains over several consecutive days significantly increased the creek current, risking the survival of clutches and tadpoles who depend on slow water for their development (Rico *et al.*, 2004). The unpredictability of temporary streams as places to reproduce can be compensated for by the length of the reproductive season (Diaz-Paniagua, 1990).

The observed increase in the number of calls per male as the number of males in the chorus increased, likely re-

sults from mutual stimulation among males. In *Hyla elegans*, males calling in a group emitted longer calls, with a significantly higher number of pulses than males not interacting with their neighbours (Bastos & Haddad, 1995). However, the mutual stimulation in male *S. trapicheiroi* occurred only up to a certain limit, above which the number of calls per male was independent of the number of males in the chorus. When many males are calling in a chorus, call overlap may disrupt information necessary for species recognition and female choice (Schwartz, 1987), so it may be advantageous for males to alternate calls. However, whether the threshold of 15-20 males observed for *S. trapicheiroi* results from avoidance of signal disruption or enhancement of female choice is not clear. Regardless, variation in calling rate is responsible for most of the variation in energy expenditure by calling frogs (Bevier, 1997).

In the study area, different factors seem to regulate reproductive activity for each sex, rainfall and photoperiod for males (on a monthly basis); short-term rainfall for females. Males were more constant than females at the creek. Females appeared only 56% of the nights with males at the creek. That value is comparable to 58% for *H. elegans* (Bastos & Haddad, 1995), and 42% for *Hyla faber* (Martins, 1993), but less than 84% for *Scinax rizibilis* (Bastos & Haddad, 1999). This difference may suggest that conditions for calling are less restrictive than conditions for egg laying, but may also result from the fact that those males who stay more time calling have higher reproductive success (Godwin & Roble, 1983).

We conclude that *Scinax trapicheiroi* at Ilha Grande is predominantly nocturnal. Calling activity of males was influenced by environmental factors on a monthly basis. Female presence in reproductive sites is affected by short-term rainfall.

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CHORUS ORGANIZATION OF THE LEAF-FROG *PHYLLOMEDUSA ROHDEI* (ANURA, HYLIDAE)

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We studied the chorus organization of a population of prolonged-breeding *Phyllomedusa rohdei* at a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil. Males, females and amplexant pairs were more abundant when the pond filled up for the first time (in December 1999). We found a positive correlation between the number of males and females in the chorus, but no significant correlation between operational sex ratio and the number of males present. The number of nights that males participated in a breeding chorus was shortened. The ability to remain in breeding aggregations was not correlated with the snout-vent length of males, but it was correlated with the initial body weight. Males showed high site fidelity and some turnover between consecutive nights. Larger males predominated in the chorus when there was water in the pond, while smaller males predominated when the pond was dry. The spatial distribution of males in the pond was clustered; aggregations occurred in places with adequate oviposition sites for this species. *Phyllomedusa rohdei* was not considered a lek species mainly because male territories enclose oviposition sites.

Key words: mating system, reproductive biology, spatial distribution, temporal distribution

INTRODUCTION

Numerous aspects of the social behaviour of anuran amphibians are related to breeding activities (Wells, 1977a; Heatwole & Sullivan, 1995). In general, reproductive activities of anuran species are announced by a congregation of calling males in dense choruses in a flooded area (Wells, 1977a). The primary role of these communal displays appears to be enhancement of attractiveness through the increase in volume and in the active space of the signal (Wilson, 1975). However, at a fine scale within breeding sites, the aggregative behaviour of males can be explained by several factors (see Pfennig *et al.*, 2000, and references therein). In anurans, the mechanisms responsible for aggregative behaviour of males seem to be mainly associated with sexual parasitism (Perril *et al.*, 1978; Haddad, 1991; Pfennig, *et al.*, 2000), and/or to promote the reduction of individual's risk of predation (Ryan *et al.*, 1981; Pfennig *et al.*, 2000).

A lek is usually defined as a communal sexual display area where males congregate for the purpose of attracting and courting females and to which females come for mating (Wilson, 1975). In these areas, males do not control resources critical to females, and except for their contribution of sperm, males provide no parental care for the eggs or young (Bradbury, 1981). Bradbury (1981) listed conditions to characterize a lek species. Based on it, many anurans aggregations can be classified as leks mainly because oviposition occurs away from the immediate vicinity of the calling site of males, and males provided only sperm to female mates (e.g.

Ryan, 1985; Arak, 1988; Bourne, 1992; Bastos & Haddad, 1996).

Emlen & Oring (1977) and Wells (1977a) pointed out that the length of the breeding period influences the reproductive behaviour of the species. In general, temperature and rainfall have been considered as the most important environmental factors responsible for the length of the breeding period in anuran species (e.g. Salvador & Carrascal, 1990; Lizana *et al.*, 1994; Pombal, 1997). Nevertheless, the specific environmental conditions that dictate chorus attendance by males and females in breeding sites have rarely been investigated (see Sinsch, 1988; Ritke *et al.*, 1992; Henzi *et al.*, 1995; Murphy, 2003 for exceptions).

Either in prolonged or explosive breeders (*sensu* Wells, 1977a), the number of nights that a male is present in breeding aggregation (male chorus tenure) is quite abbreviated with respect to the length of the breeding season (Ryan, 1985; Murphy, 1994a). Explanation of this pattern has centered on energy limitations to sustain the high energetic cost of calling activities (Bucher *et al.*, 1982; Murphy, 1994b; Given, 2002). More specifically, some studies showed that the energy reserves obtained before the breeding period were responsible for male chorus tenure (e.g. Green, 1990; Murphy, 1994b). Nevertheless, in spite of this high tendency to non-attendance, every night new males come to breeding sites renewing the chorus from night to night. In this sense, males may present a high (Dyson *et al.*, 1992 – *Hyperolius marmoratus*) or a low turnover (Gerhardt *et al.*, 1987 – *Hyla cinerea*).

In this paper, we examine several aspects of chorus organization of *Phyllomedusa rohdei* Mertens focusing mainly on (1) the temporal and spatial distributions in both sexes, (2) the pattern of dispersion (uniform, random, or clumped) of males, and its possible causes, (3)

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the influence of environmental variables (pond depth, air temperature and rainfall) on attendance of males, females and amplexant pairs in the pond, (3) the number of nights that males and females spent in breeding aggregations, and (4) the occurrence of site fidelity displayed by males, as well as lek behaviour based on Bradbury's conditions.

MATERIALS AND METHODS

STUDY SITE AND GENERAL FIELDWORK

A population of *Phyllomedusa rohdei* was studied at Palmital (22°50'S; 42°27'W), Municipality of Saquarema, State of Rio de Janeiro, south-eastern Brazil, from July 1999 to July 2000. The study site was a temporary pond of approximately 170 m². The pond has an approximately rectangular shape, having in its central area a small portion of land covered by vegetation which was connected to the mainland by a narrow pathway. The fieldwork was carried out monthly when the pond was dry (from July 1999 to November 1999 and from March 2000 to July 2000), and fortnightly when the pond was flooded (from December 1999 to February 2000). Each trip consisted of one to seven consecutive nights of study. We monitored the pond for a total of 411 hours in 84 visits. Field observations were conducted from 1700 hr until 2400 hr, depending on the activity of leaf-frogs.

To evaluate the level of site fidelity of males, each segment of the pond was divided into three parts of approximately 3 m wide. Individuals captured within the same area on at least two consecutive nights were considered to exhibit site fidelity. For all collected individuals we measured the snout-vent length (SVL) to the nearest 0.1 mm with caliper ruler and weighed individuals to the nearest 0.05 g with a Pesola® spring scale. We marked the leaf-frogs individually by toe clipping (Martof, 1953). The opposable fingers (numbers 100 and 800) and toes (numbers 1 and 10) were not clipped.

SURVEY OF SPATIAL DISTRIBUTION

By categorizing two physical characteristics of the pond margin (vegetation cover classified as dense or scarce; and availability of oviposition sites as with or without oviposition sites), we recognized four microhabitats: dense vegetation with oviposition sites (DWS); dense vegetation without oviposition sites (DWS); scarce vegetation with oviposition sites (SWS) and scarce vegetation without oviposition sites (SWS). Adequate oviposition sites for *P. rohdei* were defined as leaves of a width equal to or larger than the width of a female and that could be folded by them (i.e. leaf width did not exceed the the width of a female with its arms stretched). The microhabitat DWS was the largest, representing 41.8% from the pond edge (DWS 18.5%; SWS 11.9%; SWS 27.9%). The vegetation in the middle of pond and beyond the microhabitats area was not considered. The individuals were only observed on the edge of the pond. In December 1999 the mean

pond depth was 70.4±9.8 cm ($n=11$); in January 2000, 27.8±9.5 cm ($n=6$) and in February 2000, 14.1±1.5 cm ($n=5$). The air temperature was recorded in the study area at 1.5 m high. Pluviometric data (amount of rain for the month) were recorded at the Estação Rio Mole (22°51'S; 42°33'W), located approximately 10 km from study area. We performed a multiple regression analysis to assess the influence of pond depth, air temperature, and rainfall on number of males, females, and amplexant pairs using a forward stepwise method, allowing an evaluation of the relative contribution to attendance of both sexes and amplexant pairs of the three specific environmental variables (pond depth, air temperature and rainfall).

Over five nights at 2100 hr (time of the highest calling activity of males), we intensively searched for males at the calling sites. The individual positions of calling males were plotted in a schematic map, where the spatial distribution of males was determined using a Poisson distribution test. We used two methods (see Brower & Zar, 1984) to verify whether males were randomly distributed: (1) the "variance-to-mean ratio", where we calculated a χ^2 value as the sum of squares of the samples divided by the sample mean. A ratio (or coefficient of dispersion) less than 1.0 indicates a uniform distribution, and a ratio greater than 1.0 indicates clumping; and (2) the "goodness-of-fit test": to test if the observed frequencies deviate significantly from the frequencies expected by the null hypothesis of randomness, we employed a χ^2 test computed as $\sum(f - F)^2 / F$, where f is an observed frequency and F is its associated expected, or hypothesized, frequency.

Field methods and statistical procedures are taken primarily from Brower & Zar (1984) and Zar (1999). Non-parametric statistical tests were employed when assumptions of normality were violated. Descriptive statistics are given as mean ±SD for parametrical data and medians, range for non-parametrical data.

RESULTS

POPULATION STRUCTURE

We marked a total of 143 males and 69 females during the study. Females (SVL 44.4 mm, range 37.5-50.2 mm, $n=69$; mass 4.10 g, range 2.45-6.60 g, $n=57$) were longer (Mann-Whitney $U = 330.0$, $P < 0.01$) and heavier ($U = 579.0$, $P < 0.01$) than males (SVL 38.4 mm, range 25.0-45.0 mm, $n=143$; mass 2.95 g, range 1.50-4.40 g, $n=121$).

Males were observed calling across the year (from July 1999 to May 2000). However, only in December 1999 did the number of males and females increase quickly and only between December 1999 to March 2000 did we observe amplexant pairs (Fig. 1). Chorus duration and number of males calling during the night were greater in the months when the pond was flooded (December 1999, January and February 2000). The nocturnal activity peak of males (time of the greatest number of males calling) was at 2000 hr when the pond

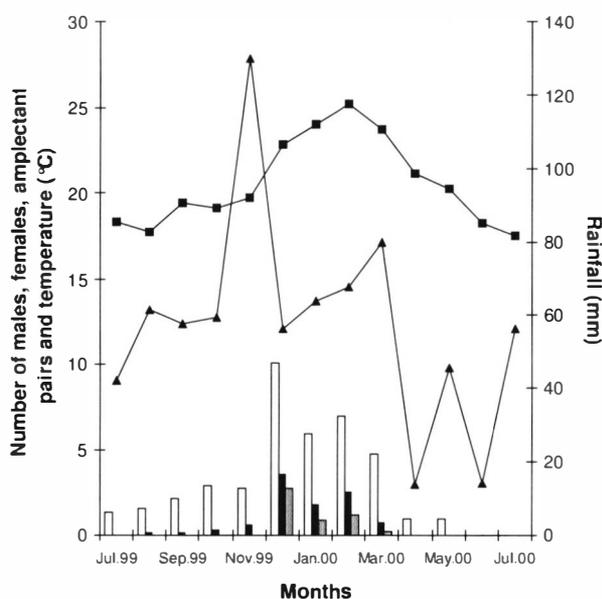


FIG. 1. Number of males (white columns), females (black columns), and amplexant pairs (dotted columns) of the *Phyllomedusa rohdei*, and air temperature (squares) and rainfall (triangles) at a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil from July 1999 to July 2000.

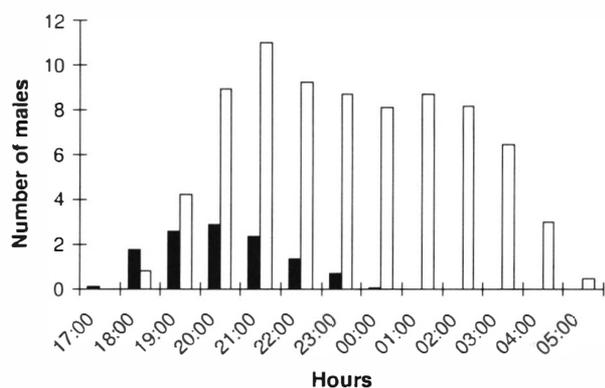


FIG. 2. Mean number of calling males of *Phyllomedusa rohdei* along the night in the months of dry pond (black columns) and in the months of flooded pond (white columns) at a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil.

was dry and at 2100 hr when the pond was flooded (Fig. 2). Males of *P. rohdei* were found over the vegetation close to water (vertical distribution: mean = 45.7±32.6

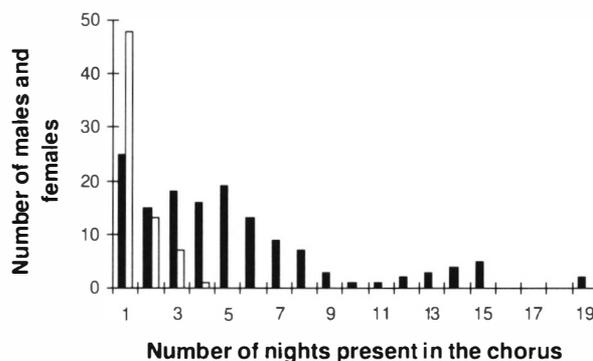


FIG. 3. Number of nights that individual males (black columns) and females (white columns) of *Phyllomedusa rohdei* attended a pond chorus between July 1999 and March 2000 in a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil.

cm from the ground, $n=155$) and on the pond's edge (horizontal distribution: mean = 40.3±23.3 cm from water, $n=112$).

The number of males found in 19 nights ranged from 8 to 61 (mean = 26.6±14.3) and the number of females ranged from 0 to 10 (mean = 4.6±2.9). The operational sex ratio (OSR: number of reproducing females/ number of reproducing males) in the population of *P. rohdei* was highly male-biased (mean = 0.19±0.10, $n=19$ nights). The number of females was positively correlated with the number of males in the chorus (Pearson correlation; $r=0.7$, $P<0.05$, $n=19$ nights), however the OSRs were not significantly correlated with the number of males present in the chorus ($r=-0.32$, $P>0.05$, $n=19$ nights), indicating that females were not proportionally more abundant in larger choruses. Correlation matrices and the multiple regression analysis are presented in Table 1. In general, the results of correlations (Pearson correlation coefficient) between pond depth (PD), air temperature (AT), rainfall (RF) and number of males (NM), females (NF) and amplexant pairs (AP) and multiple regression analyses were similar. The PD and AT were significantly correlated with NM, NF, and AP. Surprisingly, the RF was not significantly correlated with either of dependent variables. An evaluation of Beta coefficients and partial correlations between these variables reinforced the relative contribution of PD and AT in predict the NM and NF, and only PD for AP, when controlling the effect of other variables.

TABLE 1. Correlations (Pearson correlation coefficient) between pond depth (PD), air temperature (AT), rainfall (RF), and number of males (NM), number of females (NF), and amplexant pairs (AP), at a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil, and results of forward multiple regression analyses [Beta coefficient (Beta) and partial correlation (pc)].* $P<0.05$

	Correlations (r)			NM		NF		AP	
	NM	NF	AP	Beta	pc	Beta	pc	Beta	pc
PD	0.87*	0.80*	0.97*	0.69*	0.89*	0.51*	0.82*	0.90*	0.96*
AT	0.73*	0.82*	0.59*	0.35*	0.69*	0.54*	0.84*	0.13	0.48
RF	0.27	0.31	0.05	0.18	0.48	0.19	0.52	—	—
Regression results				$R^2=0.90^*$ $F_{3,9}=28.16$		$R^2=0.91^*$ $F_{3,9}=30.0^*$		$R^2=0.96$ $F_{2,10}=107.80$	

TABLE 2. Percentage of males (M), females (F), and amplexant pairs (AP) of *Phyllomedusa rohdei* present in each microhabitat (Mi) in a temporary pond in Saquarema, State of Rio de Janeiro, southeastern Brazil. M/Mi: % of males/% of length of microhabitat; F/Mi: % of females/ % of length of microhabitat; P/Mi: % of amplexus pair / % of length of microhabitat.

DWS: dense vegetation with oviposition sites; DWoS: dense vegetation without oviposition sites; SWS: scarce vegetation with oviposition sites; SWoS: scarce vegetation without oviposition sites.

Microhabitat	Males	M/Mi	Females	F/Mi	Amplexant pairs	AP/Mi
DWS	68.71%	1.65	70.21%	1.69	78.73%	1.89
DWoS	5.59%	0.30	9.57%	0.52	4.25%	0.23
SWS	15.27%	1.28	14.89%	1.25	14.89%	1.25
SWoS	10.43%	0.37	5.32%	0.19	2.13%	0.08
Total number	537 males	—	94 females	—	47 pairs	—
Nights sampled	14		17		17	

The male chorus tenure (Fig. 3) was abbreviated (4.0, range 1.0-19.0, $n=143$), representing 6.2% of the length of the study season (84 nights sampled). There was no correlation between SVL of males and the number of nights in the chorus (Spearman Rank-correlation; $r_s=0.15$, $P>0.05$, $n=143$), but we found a positive correlation between initial mass of males (mass observed on the first night in the chorus) and the number of nights in the chorus ($r_s=0.31$, $P<0.01$, $n=120$).

Females attended (1.0, range 1.0-4.0, $n=69$) fewer nights than males in the chorus ($U=1436.0$, $P<0.01$). About 70% of females spent a single night in the pond (Fig. 3). Females were present in the chorus on 26 nights (37.7% of the duration of this study, $n=69$ nights sampled). Amplexant pairs were found in 18 nights (26.1% of total). The mean number of days between two recaptures of females from December 1999 to March 2000 was 29.1 nights \pm 28.4 ($n=20$). Of 12 females which returned to the breeding site with intervals above 20 nights, about 70% were first captured in December 1999 (25% in January 2000, and 5% in February 2000). Only three females mated twice; the intervals between the ovipositions were 26, 32, and 60 days (these females first mated in December 1999). The number of eggs deposited by one of these females in the first capture was 140 eggs and in the recapture were 104.

About 45% of males (range 20-70%; $n=12$ pairs of two consecutive nights) present in one night were present in the next night. On average, the chorus on any night was formed by 40% (range 10-80%) of males present in the previous night and 60% (range 20-90%) of males that did not participated in the chorus of the

previous night. The degree of site fidelity displayed by males was high. Nearly 70% of males were found in the same place they occupied in the previous night. Individual males remained for eight nights within the same area. Males that displayed site fidelity were not significantly larger (mean = 39.0 \pm 2.4 mm, $n=76$) than males that moved out to other sites on the following night (mean = 38.5 \pm 2.5 mm, $n=39$, $t=0.95$, $P=0.34$).

The mean body size of males that were present during the dry pond periods (July 1999 to November 1999 and March 2000) (38.0 mm, range 25.0-45.0, $n=70$) was significantly smaller ($U=5604$; $P=0.0004$) than that of males that were present at the pond when it had water (December 1999, January and February 2000) (38.6 mm, range 27.1-45.0, $n=223$). The mean SVL of males among the months with water in the pond did not differ significantly (Kruskal-Wallis test: $H_{(2, n=223)}=0.38$, $P=0.83$; Median test: $\chi^2=0.25$, $df=2$, $P=0.88$).

SPATIAL DISTRIBUTION

The number of males, females, and amplexant pairs observed varied among each microhabitat (Table 2). There were more males, females, and amplexant pairs in the DWS than in other microhabitats. In these places, males performed their breeding activities, ousting intruders, attracting females, and mating. In general, the spatial distribution of males in the pond was clustered (Table 3). Both methods showed a tendency towards a clumped distribution pattern, except for two nights (21 and 29 December 1999). The mean SVL of males of the microhabitats with oviposition sites (39.0 mm, range 27.1-45.0, $n=437$) did not differ ($U=21207.5$, $P=0.88$)

TABLE 3. Coefficient of dispersion (CD, variance-to-mean ratio) and goodness-of-fit test for males of *Phyllomedusa rohdei* attending choruses at a temporary pond in Saquarema, State of Rio de Janeiro, south-eastern Brazil over 5 nights of breeding activity with different chorus size, as indicated by the Poisson distribution. * $\chi^2_{0.05, 186}=216.55$ (critical values of the chi-square distribution), † $P < 0.05$.

Date	Variance-to-mean ratio			Goodness-of-fit
	Chorus size	CD	χ^2_1 *	χ^2_2 , P -level (df)
11/12/1999	21	1.40	260.39†	1000.35, $P=0.001$ (3)
13/12/1999	27	1.35	250.75†	333.10, $P=0.001$ (3)
21/12/1999	30	1.25	232.42†	4.78, $P=0.19$ (3)
29/12/1999	32	1.18	218.61†	1.48, $P=0.48$ (2)
2/01/2000	45	1.30	242.38†	105.79, $P=0.001$ (3)

from the mean SVL of males of the microhabitats without oviposition sites (39.0 mm, range 26.0–42.6, $n=98$).

DISCUSSION

Despite the apparent disorder in breeding sites of *Phyllomedusa rohdei*, chorus exhibits some organization. The studied population exhibited typical patterns of prolonged breeders (*sensu* Wells, 1977a), having a long breeding season with relatively low male densities, asynchronous arrival of females at the breeding pond, and a strongly male-biased operational sex ratio. Although calling activities were not restricted to the rainy season, males were more abundant and allocated more time during it. We did not detect changes in the size distributions of individuals within a breeding season as observed for other species by several authors (e.g. Howard, 1978 – *Rana catesbeiana*; Sullivan, 1987 – *Bufo woodhousei*; Salvador & Carrascal, 1990 – *Bufo calamita*, *Hyla arborea*, *Pelobates cultripes*, *Rana perezi*; Lizana *et al.*, 1994 – *Pelobates cultripes*). However, compared to the dry period, larger males were more frequent in the three months when there was water in the pond. Chorus attendance by small males before or during late breeding season could reduce competition and possible injuries with larger males (Salvador & Carrascal, 1990; Lizana *et al.*, 1994). Considering the short period in which the breeding sites are available and consequently the restricted opportunity for reproduction, presumably only larger males would be successful in reproductive aggregations, if territorial defence is important.

Phyllomedusa rohdei males showed significant clumping within breeding ponds. Our results suggest that aggregations by *P. rohdei* males occur, at least in part, because they preferentially cluster in areas through which the largest number of females is likely to pass. This behaviour resembles the hotspot model which predicts that aggregations are formed by the product of attempts of males to display courtship behaviour in certain locations such as foraging, nesting or roosting sites where females may be found more easily and more abundantly (Bradbury & Gibson, 1983; Beehler & Foster, 1988). These areas preferred by both sexes had adequate oviposition sites. Yet, if microhabitats with oviposition sites afford more mating success than others (in this study: without oviposition sites), so the selection would favour larger and stronger males in the microhabitats with oviposition sites. Despite what has been demonstrated in some studies (e.g. Wells, 1977b; Howard, 1978), our results do not corroborate this hypothesis. The difference in male size between microhabitats with and without adequate oviposition sites was not significant, suggesting that larger males do not predominate in better areas.

Approximately half of the males present on any one night did not participate in the chorus of the previous night, a medium turnover rate when compared with other species (Gerhardt *et al.*, 1987; Dyson *et al.*, 1992).

The chorus tenure of *P. rohdei* males at the breeding site was shortened with respect to the length of the breeding season. This low attendance in choruses has been also observed in several species (e.g. Ryan, 1985; Murphy, 1994a). Since there was a positive correlation between initial mass of males and number of nights present in the chorus, we suggest that energy reserves accumulated before or during the beginning of the chorus can limit male attendance. A similar conclusion was found by Green (1990) and Murphy (1994b). Although chorus tenure of males had been abbreviated, they had greater attendance than females, on average, almost four times as many nights. Woodward (1984) suggested two reasons to explain why males spend more nights in the pond than females: (1) it is harder for a male than a female to obtain a mate and (2) males can mate more times than females in breeding season.

The absolute number of females arriving at the breeding site was correlated with chorus size, but larger choruses did not attract proportionally more females per male. Several studies have found similar results (e.g. Arak, 1988; Dyson *et al.*, 1992; Bastos & Haddad, 1996). Three possible explanations for the positive correlation between the number of males and females in the chorus are: (1) large choruses are more audible, therefore attracting more females (Wells, 1977a), (2) female preference for larger aggregations: females prefer to select males from larger choruses because they provide a greater range of choice (Bradbury, 1981; Ryan *et al.*, 1981) or (3) the movement to a pond is triggered by the same climatic factors in both sexes (Arak, 1988; Sinsch, 1988; Murphy, 2003). Vocalizations of *P. rohdei* males in breeding aggregations are somewhat infrequent, with long silent intervals between calls, which there is no a loud chorus. Perhaps this calling behaviour decreases the ability of females to estimate the chorus size through chorus intensity (Wells, 1977a; Bradbury *et al.*, 1981; Henzi *et al.*, 1995), suggesting that the first hypothesis above can be discarded or simply females appear not to assess the number of calling males by the intensity of signal output of a chorus (Murphy, 2003). Although the number of environmental variables recorded was somewhat restricted compared to other studies (e.g. Sinsch, 1988; Henzi *et al.*, 1995; Murphy, 2003), our data indicate that the movement of males and females to the pond can, in part, be explained by the influence of environmental variables – specifically, pond depth and air temperature. Pond depth can be considered an indirect measure of previous rainfall. In fact, more precipitation was registered in November 1999 (last days). Hence, the apparent lack of the influence of rainfall on reproductive activities of *P. rohdei* is misleading, because it was only after rain (December 1999) individuals moved to pond. In light of the data, the second explanation should not be discarded.

Twenty out of 69 females (29%) were observed twice at the breeding site. From this total, only three females mated twice. About 70% of females observed twice at

the breeding site were first captured in December 1999. The potential to breed twice in *P. rohdei* (and in other anurans with prolonged breeding) may be determined by the early arrival in the breeding season, in order to have sufficient time to produce another clutch. According to Telford & Dyson (1990), a possible constraint on anurans producing more than one egg clutch during a single breeding season is the time required to acquire sufficient energy to produce a new clutch. Thus, the ability of females to arrive early in the breeding season may be an adaptive character, especially in temporary habitats, where this behaviour may be associated with increased reproductive success.

The presence of an individual at the same position in the pond (site fidelity) has been considered as territorial behaviour (Wells, 1977b, 1978; Roithmair, 1994), which is related to competition for diverse limited resources such as female and oviposition, calling, and feeding sites (Wells, 1978; Martins *et al.*, 1998). On average, 70% of males of *P. rohdei* stayed in the same area in consecutive nights. In *P. rohdei*, these areas may function as courting and oviposition sites, which would explain the permanence of males in such areas and the vigorous agonistic actions of the resident males toward intruders (Wogel *et al.*, 2004).

Finally, according to Bradbury's (1981) conditions, *P. rohdei* was not considered to exhibit a lek mating system simply because females usually laid eggs at the same place where courtship took place. In other words, male territories enclosed oviposition sites.

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CHANGES IN COMMUNITY COMPOSITION, HABITATS AND ABUNDANCE OF SNAKES OVER 10+ YEARS IN A PROTECTED AREA IN ITALY: CONSERVATION IMPLICATIONS

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The snake fauna of different habitat types was studied in a protected Mediterranean area of central Italy ('Canale Monterano' in the Tolfa Mountains, province of Rome) during the period from August 2002 to September 2003. The collected data were compared to those collected at the same study area over 10 years before, and published by Luiselli & Rugiero (1990). We captured eight different species (seven colubrids and one viper), six of which were observed in the earlier study. During both surveys, the most common species was *Coluber viridiflavus*, followed by *Vipera aspis*. In the 2002-2003 survey there was a slight increase in the value of the species diversity index but a remarkable increase in the value of species dominance index (due especially to the proportional higher abundance of *C. viridiflavus*). We used multivariate statistics, Pianka and Czechanowski overlap indices and Monte Carlo simulations on the habitat use states during the two survey periods to document whether the various species modified their habitat preferences between surveys. In terms of habitat preferences, all these indicators showed that there were substantial interspecific differences but that the species-specific preferences remained the same over the two survey periods. There was a decrease in the abundance of *Elaphe longissima* and, to a lesser degree, *V. aspis*, caused especially by clearing brush at an archaeological site where these snakes were very common over 10 years ago. This is potentially relevant in conservation terms, as in places such as Europe, where many of the protected areas are set aside for archaeological or historical (as opposed to biological) reasons, management to maintain sites or improve access may be detrimental to native species. The various habitats differed in their conservation value for snakes. Appropriate management of the 'dry-stone walls and oak forests' habitat-mosaic appeared especially important for the conservation of this snake community, and the same may well be true for many other areas in Mediterranean central Italy.

Key words: central Italy, habitat selection, historical changes, Mediterranean protected areas, Serpentes

INTRODUCTION

The study of habitat selection is important if we are to understand the evolutionary ecology and conservation of snakes (Reinert, 1997). The concept of habitat specificity of organisms has played a major role in the formulation of general theories on species diversity and the organization of living communities (e.g. Rosenzweig, 1981). Indeed, most (perhaps all) snake species use their available habitats in a non-random way, and this non-random distribution of habitats does not seem to be the immediate result of differential survival in adjacent habitats (Reinert, 1997).

Habitat preferences in snakes may vary intraspecifically (e.g. Reinert, 1984; Shine, 1986; Burger & Zappalorti, 1989; Luiselli *et al.* 1994, etc), geographically (e.g. Sweet, 1985), seasonally (e.g. Seigel, 1986; Reinert, 1997), and even ontogenetically (e.g. Beatson, 1986; Reinert, 1997). Most studies have examined the patterns of habitat preferences in snakes over short timeframes, and long-term studies are rarer. When long-term studies are logistically problematic,

short-term studies repeated over 10 or more years can at least give some indications of the temporal variation in patterns of habitat preferences of snakes within a given study area.

Over 15 years ago, Luiselli & Rugiero (1990) published a study on the habitat selection of snakes at a Natural Park in northern Latium (Canale Monterano in the Tolfa Mountains, central Italy) which was considered by the authors of great scientific interest because of its herpetofaunal richness, being inhabited by six sympatric species of snakes and several other reptiles as well. As a step toward the evaluation of the biodiversity status of this protected area after more than 10 years of protection, the Park's Authorities launched a novel field study on the snake communities of this territory. This new project provided the opportunity to compare habitat preferences and relative abundance of the snakes of this area after a relatively long time-span (>10 years after the earlier study). The results obtained are presented in this paper. In particular, we aim to address the following questions: (1) Is the snake community composed of the same species? (2) Have the various species modified their habitat preferences over the intervening years? (3) If so, can the observed changes be linked to modifications in habitats available? (4) Has the relative

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abundance of the various species change over the study period? (5) If so, what are the main factors affecting the relative abundance of species? (6) What are the main conservation implications of this study both at the local level of the study area and at the general level of the Mediterranean ecosystems in central Italy?

MATERIALS AND METHODS

STUDY AREA

The field study was carried out at the 'Riserva Naturale Regionale Monterano', situated about 50 km north of Rome (Tofa Mountains, Latium, central Italy). The study area was exactly the same as that used by Luiselli & Rugiero (1990) for their field study. The study area was officially protected since the end of the 1980s, and during the 1990s it was also considered a European Community Interest Site under the program Natura2000 of the European Union (site code: IT6030001, 'Fiume Mignone - medio corso'). This hilly area, with a surface of 488.3 ha and ranging in elevation from 150 to 250 m a.s.l., was characterized by a patchy mosaic of habitat types associated with such disparate vegetation as *Ulmus*, *Populus*, *Salix*, *Alnus*, *Fraxinus angustifolia* in the riparian areas; *Quercus cerris*, *Q. pubescens*, *Ostrya*, *Carpinus orientalis*, *Acer monspessulanum*, *Cercis*, *Paliurus*, and *Castanea sativa* as mixed woodlands, and *Callitricho-* and *Thero-Brachypodetalia* and *Brometalia* in the grassy pastures (Spada, 1977). At a elevated spot, the ruins of an ancient town (Monterano) dominate the area. These ruins were completely surrounded by dense bushes of *Rubus* spp., *Rosa canina*, *Crataegus monogyna*, and *Cytisus scoparius* up to seven years ago, but since then they were almost entirely cleared under an archeological-historical programme supported by the European Union. The climate of the study area was Mediterranean-temperate, with cold winters (usually without snow), rainy spring and autumn, and dry and hot summer (hypomesaxeric subregion [type B] according to Tomaselli *et al.*, 1973).

PROTOCOL

The field study was conducted at the various habitat types available in the study area from August 2002 to September 2003. Fieldwork was conducted under all climatic conditions, and a total of 359 man-hours were spent in the field. In the various appropriate habitats (see below), we searched for snakes by time-fixed routes. Each route in each habitat type - surveyed by two independently walking searchers - was 60 min long. During a day with optimal weather (sunny and moderately warm), we typically carried out at least one time-fixed route in at least five different habitats, and the sequence of habitat types surveyed varied randomly in such a way to maintain a relatively constant field effort in each habitat type. Although it was impossible to standardize exactly the field effort in each habitat type in relation to the relative availability of that habitat type in the land-

scape, every possible effort was done to minimize eventual biases among habitats.

The following habitat types were considered: (1) mixed oak woodland (WDS); (2) grassy pastures (GPS); (3) bushlands with *Cytisus scoparius* as the prominent taxon (CTS); (4) streams 'Mignone' and 'Fosso del Bicione' and their banks (STR); (5) ponds situated at the locality called 'Mercareccia' (PON); (6) dry-stone walls (SWL); (7) cultivations around the main town (CUL).

Snakes were captured by hand, often while they were hiding under cover. We spent most of the time scanning for snakes (about 85% of the time) rather than turning cover (about 15% of the time). Exact locality and the habitat data were recorded at each capture site. Snakes were measured for snout-vent length (SVL) to the nearest 1 mm, weighed to the nearest 1 g on an electronic balance, and individually marked by ventral scale clipping for future identification.

Field data coming from this study were compared with the dataset collected by Luiselli & Rugiero (1990) in the late 1980s. In this regard, we not only used the data available in the original paper by Luiselli & Rugiero, but also re-analysed data which are available in the field notebooks relative to that study, but eventually not published in the original study (thanks to L. Rugiero, for cooperation in this regard). The research protocol employed by Luiselli & Rugiero (1990) was nearly identical to that described above.

In this study we considered the site of capture of each snake specimen as indicative of its habitat. However, the site of capture and the actual habitat are not strictly equivalent. Capture sites are typically those used for basking, mainly during digestion or sloughing, but most of the time the snake is not exposed to capture, i.e. it is invisible and consequently its habitat is unknown.

Vouchers of all the species are stored, in alcohol, in the collections of the 'Riserva Naturale Regionale Monterano' (Canale Monterano, Rome).

STATISTICAL ANALYSES

To avoid pseudoreplication of data (Hurlbert, 1984; Mathur & Silver, 1980) habitat type was recorded only once from each individual (i.e. it was not recorded in re-captured individuals).

Having counted and recorded all the sighted/sampled specimens, we calculated species diversity and dominance indexes of each habitat type (but see Hubalek, 2000, for an evaluation of the limits of these indexes which are sensitive to sample sizes). These calculations were made for all months of research pooled, because the sample sizes were not enough to evaluate these indexes month-by-month. Species diversity (D_{mg}) was calculated using Margalef's Diversity Index (Magurran, 1988):

$$D_{mg} = (S - 1) / \ln N$$

where S is the number of species and N is the total number of individuals sampled in each zone. Species

dominance (d) was assessed using the Berger-Parker Index (Magurran, 1988):

$$d = N_{\max} / N$$

where N_{\max} is the total number of individuals of the most abundant species sampled in the zone. According to Magurran (1988), an increase in the value of $1/d$ (the reciprocal of the Berger-Parker index) indicates an increase in diversity and a decrease in dominance. For calculating the similarity in habitat use of the various snake species between the two survey periods, we calculated the overlap indices of Pianka (1973) and Czechanowski (Feinsinger *et al.*, 1981) for the habitat type frequency use of the six species observed in both the Luiselli & Rugiero's (1990) and the '2002-2003' surveys. Pianka's formula for species j and k , with resource utilizations p_{ji} and p_{ki} , is:

$$O_{j,k} = O_{k,j} = \sum p_{2i} \times p_{1i} / \{ \sum (p_{2i}^2 + p_{1i}^2) \}^{1/2}$$

In this formula the values range from 0 (no overlap) to 1 (total overlap). Czechanowski's formula for species 1 and 2, with resource utilizations p_{1i} and p_{2i} , is:

$$O_{1,2} = O_{2,1} = 1.0 - 0.5 \times \sum |p_{1i} - p_{2i}|$$

Graphically, this index corresponds to the intersection of the utilization histograms of the two species, and also ranges from 0 (no overlap) to 1 (total overlap) (Gotelli & Entsminger, 2000). We calculated these indices using the program 'EcoSym 700' (Gotelli & Entsminger, 2000). We performed a cross-tabulation on those frequencies to determine where differences in habitat types used existed for each species between the two survey periods. By means of the 'EcoSym' package, we performed Monte Carlo simulations to create 'pseudocommunities' (Pianka, 1986) and statistically compared the derived patterns with those in the actual data matrix. We used the RA3 model in 'EcoSym' to

evaluate the similarity in habitat use (= overlap); this model randomises particular resource states used by each species while retaining niche breadth. This model has been shown to have robust statistical properties for detecting non-random niche overlap patterns (or, as in our study case, similarity in resource use between survey periods of a same species; Winemiller & Pianka, 1990), and has also been successfully used previously with snakes (Laurent & Kingsbury, 2003). As we did not have a static measure of habitat type availability at the study area, we therefore used the default setting of equiprobable resource states available in 'Ecosym', exactly as done by Laurent & Kingsbury (2003). The assumption of equiprobability of resource states means in our study case that the various habitat type states (= resource states) are equally usable (= abundant) by all species in each of the two survey periods. Statistical analyses were done by 'Statistica version 6.0' for Windows PC package, with all tests being two-tailed and alpha-set at 5%, and Monte Carlo simulations were done by 'Ecosym 700' PC package. When χ^2 tests had $df=1$, the Yates' correction factor was applied.

RESULTS

APPARENT ABUNDANCE AND DIVERSITY OF SNAKES

Despite the research effort (expressed as the number of man-hours in the field) was slightly higher in the 2002-2000 survey, both the total number of snakes captured and the snake abundance (expressed as the number of snakes \times hr⁻¹) were slightly higher in Luiselli & Rugiero's (1990) surveys (Table 1). Six species of snakes were observed by Luiselli & Rugiero (1990), and eight species were observed in 2002-2003 (i.e. the six species found by Luiselli & Rugiero plus *Coronella girondica* and *Natrix tessellata*; Table 1). During both the survey periods, the most common species was *Coluber* (= *Hierophis*) *viridiflavus*, followed by *Vipera aspis* and *Natrix natrix*. All the other species were much less common in the 2002-2003 survey, but *Elaphe* (=

TABLE 1. Total number of snakes observed at the study area, and their percentage occurrence, during the two survey periods.

Species	No. of specimens (%) Luiselli & Rugiero (1990)	No. of recaptures Luiselli & Rugiero (1990)	No. of specimens (%) 2002-2003 survey	No. of recaptures (%) 2002-2003 survey
<i>Vipera aspis</i>	73 (26.4%)	39	57 (21.8%)	36
<i>Coluber viridiflavus</i>	83 (30.1%)	16	114 (43.7%)	24
<i>Coronella austriaca</i>	3 (1.1%)	0	1 (0.4%)	0
<i>Coronella girondica</i>	0	0	3 (1.1%)	0
<i>Elaphe longissima</i>	37 (13.4%)	8	15 (5.7%)	7
<i>Elaphe quatuorlineata</i>	13 (4.7%)	4	13 (5.0%)	5
<i>Natrix natrix</i>	67 (24.3%)	19	53 (20.3%)	21
<i>Natrix tessellata</i>	0	0	5 (1.9%)	0
Total sample	276	86	261	93
Field effort (man-hours)	334		359	
Snake abundance (snakes \times hr ⁻¹)	0.826		0.727	

Zamenis longissima was common during the Luiselli & Rugiero's (1990) survey. If we compare the frequency of occurrence of the various species in relation to the total number of snakes captured during the two survey periods (Table 1), it appeared that: (1) *C. viridiflavus* was significantly more abundant in the 2002-2003 survey than in Luiselli & Rugiero's (1990) survey ($\chi^2=4.56$, $df=1$, $P<0.033$); (2) *E. longissima* ($\chi^2=39.72$, $df=1$, $P<0.0001$) and *V. aspis* ($\chi^2=26.46$, $df=1$, $P<0.0001$) were significantly less abundant in the 2002-2003 survey; (3) *N. natrix* ($\chi^2=0.37$, $df=1$, $P=0.545$) and *Elaphe quatuorlineata* ($\chi^2=0.09$, $df=1$, $P<0.786$) did not show significant changes in abundance over the two survey periods; (4) *Coronella austriaca* was extremely rare during both the survey periods, but the small sample size impeded any statistical analysis.

Compared to Luiselli & Rugiero's (1990) survey, in the 2002-2003 survey there was a slight increase in the value of D_{mg} due to the addition of two species, but a remarkable increase in the value of d due to the proportional higher abundance of *C. viridiflavus* and the relative decrease in the abundance of *E. longissima* and *V. aspis* (Table 2).

HABITAT PREFERENCES

Luiselli & Rugiero's (1990) study. The original study by Luiselli & Rugiero (1990) did not analyse in full the habitats in which all the species were captured, and so we have performed a reanalysis of the original dataset (Fig. 1: *C. austriaca* was not included because of its small sample size). There were some remarkable interspecific differences in habitat type ($P<0.0001$ at χ^2 test), with *V. aspis* and *E. longissima* being linked mainly to WDS, *C. viridiflavus* and *E. quatuorlineata* to GPS and to CTS (but the former was very generalist), and *N. natrix* to STR and PON (Fig. 1). The habitat types in which we observed the higher numbers of snakes were WDS, CTS, and STR (Fig. 1). However, in WDS most of the observed specimens belonged to a single species (*V. aspis*) which was particularly abundant there, and the same was true for GPS (*C. viridiflavus*) and STR (*N. natrix*).

2002-2003 survey. The number of snake specimens observed in relation to habitat type is presented in Fig. 1B

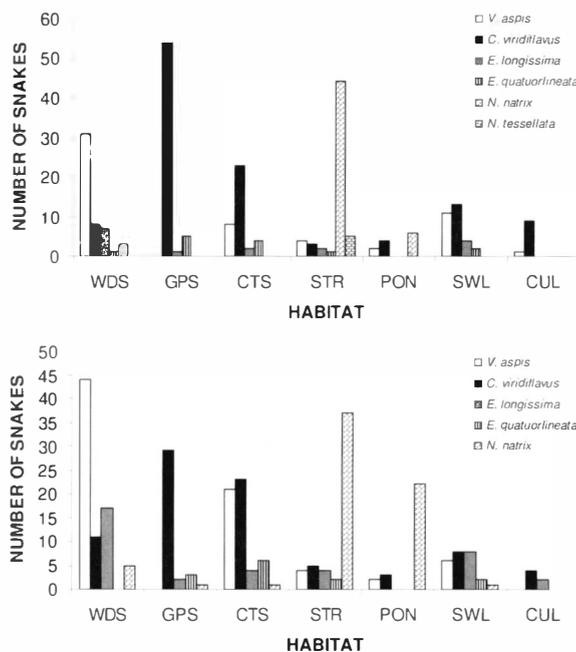


FIG. 1. Number of snakes observed at the study area in relation to the habitat of observation. Top: based on data by Luiselli & Rugiero (1990); bottom: based on data collected during the 2002-2003 survey. Symbols for habitat types: WDS, mixed oak woodland; GPS, grassy pastures; CTS, bushlands with *Cytisus scoparius* as prominent taxon; STR, stream 'Mignone' and its banks; PON, pond; SWL, dry-stone walls; CUL, cultivations.

(data for the two species of *Coronella* not included because of small sample sizes). The three specimens of *C. girondica* were of dead specimens taken by local people, so their habitat of capture was unknown. The single *C. austriaca* was captured in SWL. There were some remarkable interspecific differences in habitat type ($P<0.0001$, χ^2 test), with *V. aspis* and *E. longissima* being linked mainly to WDS, *C. viridiflavus* and *E. quatuorlineata* to GPS and to CTS (but the former was very generalist), and the two *Natrix* species to STR. Interestingly, *N. tessellata* did not occur at PON, whereas *N. natrix* did. The habitat types in which we observed the higher numbers of snakes were WDS, CTS, STR, and SWL (Fig. 1). However, in WDS most of the specimens belonged to

TABLE 2. Values of species diversity (D_{mg}) and species dominance (d) indexes at the various habitat types in relation to the numbers of snakes captured. Symbols for habitat types: WDS, mixed oak woodland; GPS, grassy pastures; CTS, bushlands with *Cytisus scoparius* as prominent taxon; STR, stream 'Mignone' and its banks; PON, pond; SWL, dry-stone walls; CUL, cultivations.

Habitat type	D_{mg} - Luiselli & Rugiero (1990)	d - Luiselli & Rugiero (1990)	D_{mg} - 2002-2003	d - 2002-2003
WDS	0.691	0.571	0.990	0.449
GPS	0.844	0.829	0.488	0.900
CTS	0.998	0.419	0.831	0.622
STR	1.012	0.711	1.226	0.746
PON	0.509	0.815	0.806	0.500
SWL	1.243	0.320	0.882	0.433
CUL	0.558	0.333	0.435	0.900
Total	0.890	0.300	1.078	0.437

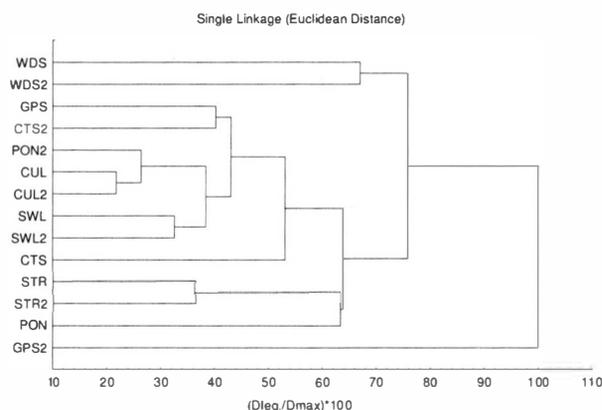


FIG. 2. Dendrogram yielded by hierarchical cluster analysis (UPGMA, standardized to 100%) of percentage composition of snake assemblages in the various habitat types at the study area, during the 2002-2003 survey and the Luiselli & Rugiero's (1990) study. Symbols for habitat types as in Fig. 1. In all cases, the symbol '2' after the habitat symbol (e.g., WDS2, GPS2, etc) indicates data from Luiselli & Rugiero (1990).

a single species (*V. aspis*), and the same was true for GPS (*C. viridiflavus*) and STR (*N. natrix*).

COMPARISONS BETWEEN THE TWO SURVEYS

D_{mg} for four habitat types differed remarkably between survey periods: the values were higher during the 2002-2003 survey in WDS and PON, but were higher during the Luiselli & Rugiero's (1990) survey in GPS and SWL (Table 2). With regard to d , there were noteworthy differences between survey periods in the values relative to habitats PON (higher in Luiselli & Rugiero's survey) and CUL (higher in the 2002-2003 survey). A hierarchical cluster analysis indicated that the snake community composition was similar between surveys in only three habitats (CUL, SWL, and STR), which indeed clustered very clearly in the UPGMA graphic (Fig. 2). These similarities can be explained as follows: (1) CUL was characterized by a very low number of species and the preponderance of *C. viridiflavus*; (2) SWL was characterized by a relatively high number of species, and the frequency of occurrence was stable; (3) STR was characterized by a great preponderance of *N. natrix*.

TABLE 3. Habitat use similarity between surveys for the various snake species assessed by calculating Pianka and Czechanowski overlap indices, and by calculating the probability that the observed indices were (1) less than expected and (2) greater than expected than those generated by Monte Carlo randomisations. Only the snake species which appeared more abundant in the study area were used for this analysis.

Snake species	Pianka's index	Czechanowski's index	P of indices being less than expected	P of indices being higher than expected
<i>Vipera aspis</i>	0.960	0.840	(Pianka = 0.8) (Czech. = 0.9)	(Pianka = 0.2) (Czech. = 0.1)
<i>Coluber viridiflavus</i>	0.953	0.827	(Pianka = 0.7) (Czech. = 0.8)	(Pianka = 0.3) (Czech. = 0.2)
<i>Elaphe longissima</i>	0.991	0.924	(Pianka = 0.8) (Czech. = 0.8)	(Pianka = 0.2) (Czech. = 0.2)
<i>Natrix natrix</i>	0.919	0.722	(Pianka = 0.8) (Czech. = 0.6)	(Pianka = 0.2) (Czech. = 0.4)

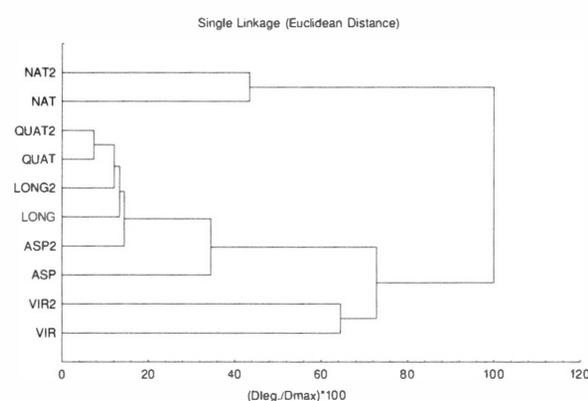


FIG. 3. Dendrogram yielded by hierarchical cluster analysis (UPGMA, standardized to 100%) of percentage use of habitat types by snakes at the study area, during the 2002-2003 survey and the Luiselli & Rugiero's (1990) study. Symbols for snake species: ASP, *Vipera aspis*; NAT, *Natrix natrix*; LONG, *Elaphe longissima*; QUAT, *Elaphe quatuorlineata*; VIR, *Coluber viridiflavus*. In all cases, the symbol '2' after the species' symbol (e.g. ASP2, NAT2, etc) indicates data from Luiselli & Rugiero (1990).

In terms of similarity of habitat use by snakes between surveys, a hierarchical cluster analysis (Fig. 3) indicated that all species were relatively consistent in terms of habitat use during the two survey periods. The highest similarity in habitat use between surveys was observed in *E. quatuorlineata* and, to a lesser degree, *E. longissima* and *V. aspis* (Fig. 3).

We investigated the habitat use similarity between survey periods of the four most abundant species (i.e. *C. viridiflavus*, *V. aspis*, *N. natrix*, *E. longissima*) by calculating Pianka and Czechanowski overlap indices and by performing Monte Carlo simulations on the resource use states during the two survey periods. Both niche indices suggested a strong habitat overlap (= similarity) between two survey periods in all four species, although the probability that the observed indices are less than expected was greater than the probability that they were higher than expected in all cases (Table 3).

The values of habitat overlap between species pairs were in general similar between survey periods, both using Pianka's and Czechanowski's indices (Table 4),

TABLE 4. Values of Pianka's (above diagonal) and Czechanowski's (below diagonal) overlap indices for the habitat type resource between the various snake species during the two survey periods.

	<i>Vipera aspis</i>	<i>Coluber viridiflavus</i>	<i>Elaphe longissima</i>	<i>Elaphe quatuorlineata</i>	<i>Natrix natrix</i>
LUISELLI & RUGIERO'S (1990) SURVEY					
<i>Vipera aspis</i>	****	0.527	0.916	0.406	0.205
<i>Coluber viridiflavus</i>	0.561	****	0.540	0.870	0.211
<i>Elaphe longissima</i>	0.697	0.499	****	0.374	0.287
<i>Elaphe quatuorlineata</i>	0.402	0.664	0.424	****	0.269
<i>Natrix natrix</i>	0.182	0.216	0.227	0.199	****
2002-2003 SURVEY					
<i>Vipera aspis</i>	****	0.287	0.970	0.380	0.185
<i>Coluber viridiflavus</i>	0.403	****	0.404	0.946	0.066
<i>Elaphe longissima</i>	0.826	0.398	****	0.509	0.285
<i>Elaphe quatuorlineata</i>	0.441	0.797	0.495	****	0.154
<i>Natrix natrix</i>	0.162	0.118	0.182	0.133	****

thus indicating that the type of habitat niche relationships between species pairs remained quite constant across the study periods. Indeed, between surveys there were no significant differences in the means of Pianka's index values ($O_{j,k}=0.460\pm 0.256$ versus 0.419 ± 0.312 , $t=0.328$, $df=18$, $P=0.746$) nor in the means of Czechanowski index values ($O_{1,2}=0.407\pm 0.196$ versus $O_{1,2}=0.396\pm 0.259$, $t=0.113$, $df=18$, $P=0.911$). Moreover, as expected Pianka's index values were significantly correlated with Czechanowski's index values ($r=0.961$, adjusted $r^2=0.919$, $n=20$, $P<0.0001$).

DISCUSSION

SPECIES COMPOSITION OF THE SNAKE COMMUNITY

All the species observed almost 15 years ago by Luiselli & Rugiero (1990) were observed again during the present study, and the species which were dominant at that time (*C. viridiflavus* and *V. aspis*) were also the most abundant species during the present study. However, in the recent survey we discovered two additional species (*C. girondica* and *N. tessellata*) which remained undetected during the earlier survey probably because of extreme elusiveness (*C. girondica*, see Agrimi & Luiselli, 1994) or because of relative rarity (*N. tessellata*). Indeed, as *N. tessellata* is easily observed in appropriate freshwater habitats (Filippi, 2000), and the numbers of observed specimens were also low during the recent survey ($n=5$, see Table 1), we hypothesize that this aquatic species may have existed at the site in low numbers for some time.

CHANGES IN HABITAT PREFERENCES

We used multivariate (UPGMA) analyses, diversity and dominance indices, Pianka and Czechanowski overlap indices and Monte Carlo simulations on the habitat use states during the two survey periods, to document

whether the various species modified their habitat preferences between surveys. All of our analyses indicated that all the species did not exhibit any evident habitat variation between surveys. We suggest that the snakes did not change their habitat use because of the relatively stable general conditions of the study area which is a permanently managed natural reserve. In addition, Pianka and Czechanowski overlap indices on habitat data between pairs of species suggested that the habitat niche relations among species remained the same between survey periods, which is also consistent with the hypothesis of relatively stable habitat conditions at the study area during the survey periods.

CONSERVATION IMPLICATIONS

It is noteworthy that two species (*E. longissima* and, to a lesser extent, *V. aspis*) declined substantially between the two survey periods. It is likely that their apparent decline depended on that they were regularly found around the ruins of the ancient Monterano at the time of Luiselli & Rugiero's (1990) study, but now the ruins have been cleared bushes for archaeological reasons, and this has probably impacted negatively on the populations of these snakes (Luiselli & Capizzi, 1997; Filippi, 2003). The negative effects of such management at an archaeological site is potentially significant in conservation terms. In places such as Europe, where many of the protected areas are set aside for archaeological or historical (as opposed to biological) reasons, land managers attempting to maintain sites or improve access to them may do real harm to native species. For instance, other potential examples of archaeological areas which are concurrently characterized by rich snake communities in central Italy are 'Vejo' (about 15 km N of Rome) and 'La Marcigliana' (about 10 km NE of Rome), and in both these areas the archaeological sites are 'maintained' through clearance of scrub. In both areas the

effects of such clearance on snake communities are now under study by us, in order to document whether there is really a conflict between the goals of archaeologists and conservation biologists in the archaeological sites which are managed in this manner. In the meantime, we urge herpetologists to report the effects of archaeological management on species-rich snake communities in other regions of Europe. Our analyses also indicated that, in terms of biodiversity value, the WDS-SWL habitat mosaic was crucial during both survey periods. The mosaic combination of these habitats should therefore be specially preserved for snake conservation, not only at the local level of the study area, but also more generally within the Mediterranean landscape, as it occupies very important fragments of the whole landscape in central Italy (Tomaselli *et al.*, 1973; Spada, 1977). SWL may be especially important during the spring, when the males of most species tend to use dry-stone walls surrounded by dense spiny bushes as corridors for mate searching (Luiselli & Capizzi, 1997, and unpublished data), whereas WDS is used primarily during the hot and dry summer, when snakes often need to retreat from high ambient temperatures (often >32-35°C). Dry-stone walls represent optimal habitat for Mediterranean snakes if they are surrounded by bushes (especially *Rubus* spp., and *Cytisus* spp.), whereas clearance of such vegetation may strongly affect the abundance of snakes if the walls are not directly removed or damaged (see Filippi, 2003). The mosaic of dry-stone walls crossing woodlands and fields are essential not only for the snakes in this study area, but also for threatened snake populations elsewhere in Italy (e.g. *Elaphe situla* in Apulia and *Vipera ammodytes* in north-eastern Italy, see Filippi & Luiselli, 2000), as well as for *E. longissima* in the Neckar-Odenwald region of Germany (Gomille, 2002). Other habitat types are also certainly important for the conservation of snake biodiversity, but probably they are less crucial than the combination of SWL and WDS in order to maintain an optimal level of stability in Mediterranean snake communities.

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ECOLOGY OF THE COLUBRID SNAKE *PSEUDABLABES AGASSIZII* IN SOUTH-EASTERN SOUTH AMERICA

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The colubrid *Pseudablabe agassizii* is a small philodryadine snake distributed in open areas in south-eastern South America. We provide information on morphology, habitat use, diel activity, diet, feeding behaviour, reproduction, and seasonal activity of this species, based on dissection of 146 specimens combined with field and captive observations. *Pseudablabe agassizii* is smaller than any other species in the Philodryadini. Females attain larger body size than males. Sexual dimorphism was also recorded for stoutness and tail length, but not for head length. Apparently, *P. agassizii* forages during the day, mainly for resting spiders in subterranean and other day-time retreats. Lycosid and other araneomorph spiders were the staple food item, but mygalomorph spiders, scorpions, and orthopteran insects were also eaten. Large spiders were subdued by venom injection, whereas smaller ones were usually swallowed alive. Ingestion of lizards is infrequent and probably represents a vestigial trait. Absence of sexual dimorphism in relative head length may be related to ingestion of small prey. The reproductive cycle of females seems to be highly seasonal with vitellogenesis occurring from the onset to the middle of the rainy season, when females are more active. Recruitment of newborns takes place at the end of the rainy season. Males show increase in testes volume in the second half of the rainy season, and mating probably occurs at the end of rainy season, when adult males are more active. At least in south-eastern Brazil, *P. agassizii* is a habitat specialist, sensitive to habitat alteration, and thus is an indicator species of environmental quality. Due to the rapid destruction of its main habitat, the Cerrado, the conservation status of this snake should be regarded as threatened.

Key words: activity, feeding, morphology, Philodryadini, reproduction

INTRODUCTION

The New World colubrids exhibit great variation in habitat, diet, and reproduction, but ecological attributes are often conserved in a particular lineage (Greene, 1997). The subfamily Xenodontinae represents the most diverse radiation of colubrids in South America (Cadle & Greene, 1993). Four genera (*Ditaxodon*, *Philodryas*, *Pseudablabe* and *Tropidodryas*) of rear-fanged snakes are grouped in the tribe Philodryadini (Ferrarezzi, 1994; Vidal *et al.*, 2000). These snakes grow to more than 600 mm snout-vent length (SVL), and all species supposedly feed on a wide range of vertebrates (cf. Vitt, 1980; Thomas, 1976; Sazima & Puerto, 1993; Marques *et al.*, 2004). However, *Pseudablabe agassizii* differs from other Philodryadini due to its small body size and specialised diet (Viñas *et al.*, 1989). This snake is relatively rare in herpetological collections, although it is widely distributed from central and south-eastern Brazil to north-eastern Argentina and Uruguay (Peters & Orejas-Miranda, 1970; Viñas *et al.*, 1989; Kiefer, 1998; Giraud, 1999; Nogueira, 2001a). Detailed ecological data are available for some species of Philodryadini (see Vitt, 1980; Sazima & Puerto, 1993; Fowler & Salomão,

1994a,b; Hartmann, 2001; Marques & Sazima, 2004), but little is known about the natural history of *P. agassizii*.

Here we provide information on morphology, habitat use, diel activity, diet, feeding behaviour, reproduction, and seasonal activity of *P. agassizii* from central, south-eastern, and southern Brazil. Except for the southern populations, specimens of *P. agassizii* in Brazilian collections were collected exclusively within the Cerrado domain. Studies on the Cerrado fauna are important and urgent given the fast rate of destruction of these natural landscapes during recent decades (Ratter *et al.*, 1997; Oliveira & Marquis, 2002).

MATERIALS AND METHODS

Our study is based on the dissection of preserved specimens from herpetological collections, and observations in the field and in captivity. We obtained data on diet, reproduction and morphology by analyses of specimens from several localities in the Brazilian Cerrado and the grasslands of southern Brazil (see Appendix 1). The Cerrado domain from central to south-eastern Brazil has a relatively homogeneous climate characterized by two distinctive seasons: a dry one from April to September, with less rainfall and lower temperatures; and a rainy one from October to March, with higher rainfall incidence and temperature (see Nimer, 1989; Sawaya, 2004). The grasslands in southern Brazil have a highly

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seasonal climate with higher temperatures from September to March, and lower temperatures from April to August (IAPAR, 1978). Rainfall is abundant throughout the year, and there is no dry season (Nimer, 1989).

We examined 122 specimens from the collections of the Instituto Butantan (IB) and Universidade de Brasília (CHUNB), as well as 24 individuals collected at the Estação Ecológica de Itirapina (São Paulo state, Brazil), totalling 146 specimens. The following data were recorded from each specimen: (1) snout-vent length (SVL; mm); (2) tail length (mm); (3) head length (0.01 mm); (4) sex; (5) reproductive maturity (males were considered mature if they had enlarged testes or opaque deferent ducts; females were considered mature if they had either oviductal eggs or ovarian vitellogenic follicles >5 mm; see Shine, 1977a,b); (6) diameter of largest ovarian follicles or oviductal eggs (0.01 mm); (7) length, largest and smallest diameters of testes (0.01 mm; testicular volume was estimated as the volume of ellipsoid, see James & Shine, 1985); (8) right deferent duct diameter close to cloaca (0.01 mm; see Almeida-Santos *et al.*, in press); and (9) stomach and/or intestine contents.

Sexual dimorphism in SVL was tested by Student's *t*-test, and sexual dimorphism in relative mass (stoutness; using the cube-root of the mass, after draining the excess of preservative liquid through ventral incisions, see Martins *et al.*, 2001), relative tail length, and relative head length were examined with one-way analyses of covariance (ANCOVA; all variables natural log-transformed), with total length, SVL, and trunk length (SVL - head length) as covariates, respectively (Zar, 1996).

Data on habitat use and seasonal activity were obtained at the Estação Ecológica de Itirapina (EEI, ca. 2,400 ha; 22°00' to 22°15' S; 47°45' to 48°00' W; elevation c. 800 m; Cerrado domain) in south-eastern Brazil. Field work at EEI was part of a study on natural history and ecology of the local snake assemblage (see Sawaya, 2004), from September 1998 to March 2002. Pitfall traps with drift fences (Greenberg *et al.*, 1994; Cechin & Martins, 2000) were installed on three protected and conserved Cerrado habitats inside the reserve: shrubby grasslands or "campo sujo" (total area: 1009.7 ha), the border of a gallery forest (1040.7 ha), and shrubby grasslands with trees or "campo cerrado" (292.7 ha). Three sample units were installed in each habitat. Each sample unit consisted of a pair of 45 m arrays, 100 m from each other, and each array had four 100-litre plastic buckets placed every 15 m, connected by a 0.5 m high fence of plastic mesh. Each day of pitfall sampling corresponded to 72 bucket-days, along 810 m of fences. We sampled a total of 293 non-consecutive days (21,096 bucket-days). Additionally, we found snakes by incidental sightings (Martins & Oliveira, 1999), with a sampling effort of 446 days of fieldwork, and sampled altered areas outside the reserve with the aid of local collectors (see Cunha & Nascimento, 1978), with 167 collector-months of sampling effort. We used the pitfall trap and incidental sightings data to test for habitat se-

lection by *P. agassizii*. We compared the observed with expected number of snakes ($n=23$) caught in each habitat, with a chi-square test (Zar, 1996), considering the differential availability (total area) of each one inside the reserve. The expected numbers of snakes in the three habitats were 9.89 in the "campo sujo", 10.12 in the border of the gallery forest, and 2.99 in the "campo cerrado".

Additional data on seasonal activity were obtained from records of individuals received in herpetological collections. We compared the observed with expected number of snakes, registered during dry and wet seasons, with a chi-square test (Zar, 1996).

Observations on behaviour and diel activity were made on a captive female of *P. agassizii* caught at the EEI (325 mm SVL), for which we recorded twenty prey encounters. The snake was housed in a 50 × 25 × 30 cm terrarium with deep soil substrate. We offered lizards (*Hemidactylus mabouia*), scorpions (*Tityus serrulatus* and *Bothriurus araguaye*), spiders (*Lycosa* sp. and *Eunoploctenus* sp.) and insects (Othoptera, Blataria) as prey. All feeding sequences were recorded with VHS and/or photographed. The captive specimen was monitored with a closed-circuit television system during 10 days for records of its diel activity.

As testes size and deferent duct diameter are also related to body size, we first use residuals of linear regressions between these variables and SVL (all natural log-transformed; see Pizzatto & Marques, 2002) to explore reproductive cycles of mature males throughout the year (see Fig. 2). These residuals are treated herein as relative testis volume and relative deferent duct diameter. We tested for differences in testis volume and duct deferent diameter, between February to March (peak of the male reproductive cycles, see below) and April to January, with one-way analyses of covariance (ANCOVA), with SVL as covariate to eliminate the body size effects on these variables (all variables natural log-transformed; Zar, 1996). Reproductive cycle of mature females was examined in terms of length of the largest ovarian follicle or oviductal egg throughout the year. Additional information on reproduction were obtained from two captive gravid females. We assessed fecundity as the number of oviductal eggs and large vitellogenic ovarian follicles (>10 mm). The relationship between maternal SVL and fecundity was analysed by linear regression (Zar, 1996). All statistical analyses were done using Statistica (StatSoft, 2003), and differences were considered significant when $P<0.05$.

RESULTS

MORPHOLOGY AND SEXUAL DIMORPHISM

Mature males averaged 261.6 mm SVL (SD=35.8, $n=35$, range=200.0-329.0), and mature females averaged 325.3 mm SVL (SD=47.2, $n=80$, range=226.0-453.0). This difference was highly significant ($t=7.14$, $P<<0.001$). The degree of sexual dimorphism (SSD) was 0.24 (*cf.* Shine, 1994). Adult females have

stouter body (ANCOVA, $F_{1,51}=5.32, P=0.03$), and shorter relative tail length than males (ANCOVA, $F_{1,107}=117.44, P<<0.001$). However, males and females have no significant differences in relative head length (ANCOVA, $F_{1,100}=1.22, P=0.27$).

HABITAT USE

From the 23 individuals for which we have habitat use data (21 caught in pitfall traps and two by incidental sightings), most snakes ($n=21$, approximately 90%) were collected in shrubby grasslands or “campo sujo”, one was found on the border of gallery forest and shrubby grasslands, and the other in shrubby grasslands with trees or “campo cerrado”. The habitat distribution of observed snakes was significantly different than expected by chance, considering the total area occupied by those three habitats ($\chi^2=22.02, P<0.001, df=2$). No snake was found in disturbed areas outside of the reserve, sampled only by local collectors and incidental sightings. In another study, between October 2003 and May 2004 (F. Spina, pers. comm.), no specimens of *P. agassizii* were found in a disturbed area (*Eucalyptus* groves) in the neighbourhood of the reserve. In that study, six sample units of pitfall traps (48 buckets along 540 m of fences) were installed, as in the present study, with 160 non consecutive days of sampling (7680 bucket-days).

DIEL ACTIVITY

Two individuals found by incidental sightings in the field were active during the day (the diel activity of snakes captured by pitfall traps could not be recorded). The captive specimen monitored was active only during

the day, moving in the terrarium mainly between 1000-1400 hr.

FOOD HABITS

Except for a lizard, all the other prey items found in the gut of *P. agassizii* were arthropods ($n=24$; Table 1). Arachnids were the most common prey, comprising 72% of the 25 items recorded. Diurnal and/or nocturnal lycosid spiders were the most common prey ($n=11$; 44%). Other prey consumed were active nocturnal spiders (Ctenidae, Titanoecidae, and Actinopodidae), one scorpion, one orthopteran, and four unidentified insects (Table 1).

FEEDING BEHAVIOUR

The captive *P. agassizii* refused lizards ($n=2$), scorpions ($n=2$), and insects ($n=2$) as prey, but accepted readily lycosid spiders ($n=14$). Spiders were usually captured by their legs, but small spiders were occasionally caught by other parts of the body. Small spiders (prey/snake mass ratio ≤ 0.014) were readily swallowed alive, being ingested cephalothorax first ($n=2$) or abdomen first ($n=6$). For large spiders (prey/snake mass ratio ≥ 0.023), the snake used alternating jaw movements to manoeuvre the prey within its mouth. These spiders were held and envenomed until they were immobile (Fig. 1A). Large spiders were swallowed cephalothorax first ($n=1$) or abdomen first ($n=5$; Fig. 1B). Snakes thus swallowed spiders more frequently abdomen first ($n=11, \chi^2=4.57, P<0.03, df=1$) than cephalothorax first ($n=3$). In eight predatory encounters the spider bit the

TABLE 1. List and number of prey found in the guts of *Pseudablabe agassizii* ($n=24$ snakes). Percentage from the total number of items ($n=25$) in brackets.

Prey type	N	(%)
INVERTEBRATES		
Araneae		
Megalomorphae		
Actinopodidae: <i>Actinopus</i> sp.	1	(4)
Araneomorphae		
Ctenidae: <i>Ctenus taeniatus</i>	2	(8)
Titanoecidae: <i>Goeldia</i> sp.	1	(4)
Lycosidae: unidentified genus	11	(44)
Unidentified	3	(12)
Scorpiones		
Bothriuridae: <i>Bothriurus araguayae</i>	1	(4)
Insecta		
Orthoptera: unidentified genus	1	(4)
Unidentified	4	(16)
VERTEBRATES		
Lacertilia		
Gymnophthalmidae: <i>Micrablepharus</i> sp.	1	(4)

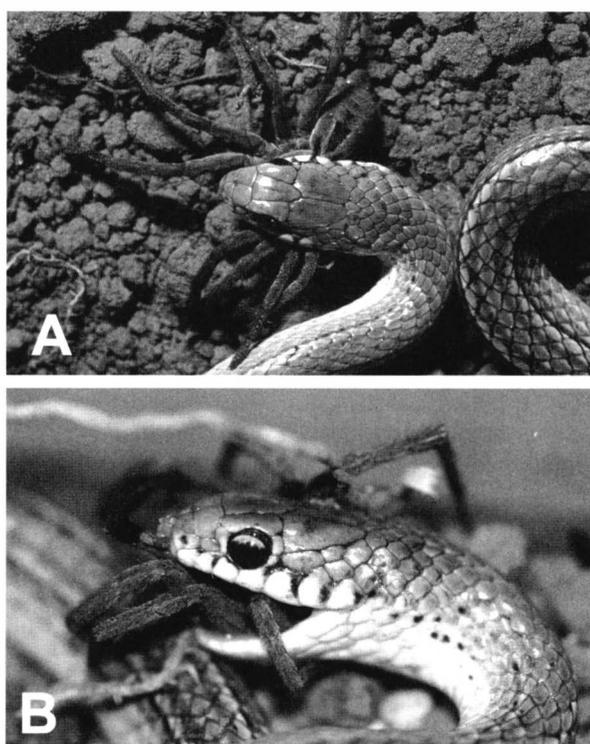


FIG. 1. *Pseudablabe agassizii* captive female: (A) holding and presumably envenoming, and (B) swallowing a spider (*Lycosa erythrognata*). Photos by O. A. V. Marques and R. Bertani, respectively.

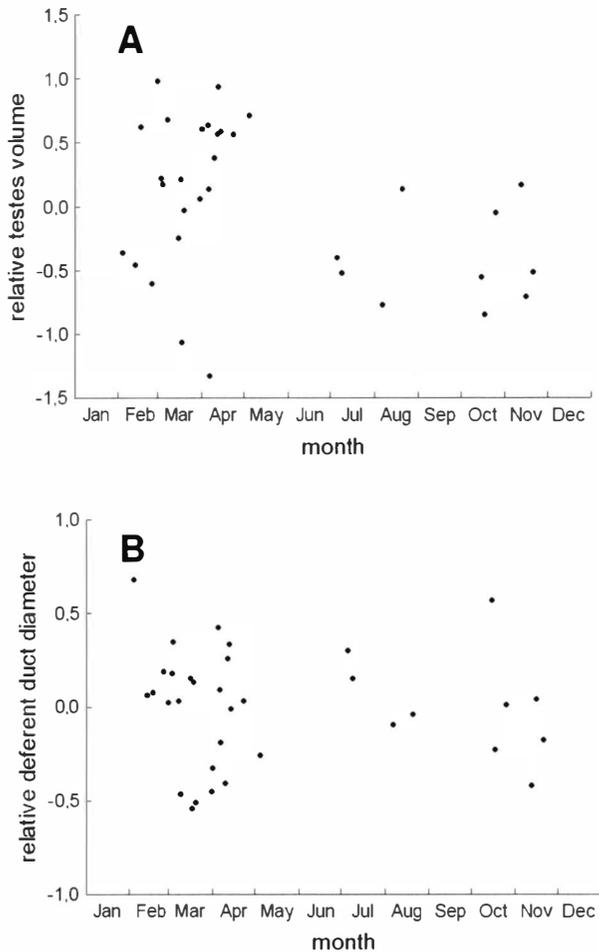


FIG. 2. Seasonal variation in relative testes volume (A), and relative deferent duct diameter (B) of mature males of *Pseudablabes agassizii* from western, south-eastern, and southern Brazil. Testes volume were significantly larger from February to April (see text).

snake's head, without any apparent damage. The time between catching the prey and the beginning of the ingestion varied from 3 to 10 min (average = 3 min 46 sec, SD=2 min 30 sec, $n=6$).

REPRODUCTION

Testes volume attained maximum values at the end of the rainy season and the beginning of the dry season, February to April (Fig. 2A), indicating a seasonal reproductive cycle in males. Relative testis volume was significantly larger at the peak of the reproductive cycle of the males (February to April) compared with other months (ANCOVA, $F_{1,33}=4.37$, $P=0.045$), but relative deferent duct diameter was not (ANCOVA, $F_{1,34}=0.01$, $P=0.92$; Fig. 2B). Females also showed a seasonal reproductive cycle (Fig. 3). Ovaries of adult females were inactive (diameter <5 mm) for much of the year, with vitellogenesis from the middle of the dry season to the middle of the rainy season (July - January), and ovulation from the end of the dry season throughout most of the rainy season (September - February; Fig. 3). One female (SVL = 460 mm) collected on 29 September 2001 laid eight eggs in captivity (7 October 2001), and another female (SVL = 333 mm) collected on 20

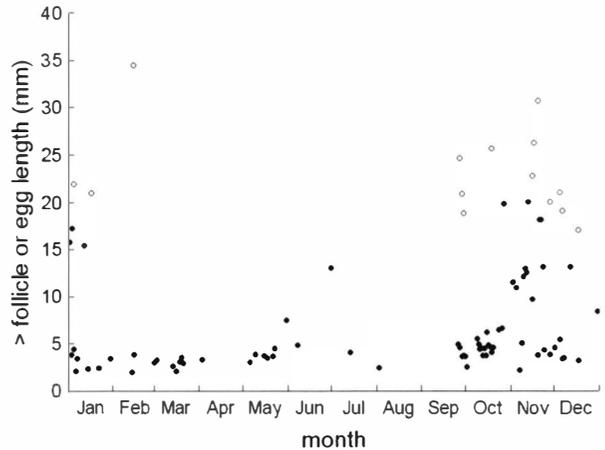


FIG. 3. Seasonal variation in diameter of the largest ovarian follicle or oviductal eggs in mature females of *Pseudablabes agassizii* from central, south-eastern, and southern Brazil. Solid circles = ovarian follicle; open circles = oviductal eggs.

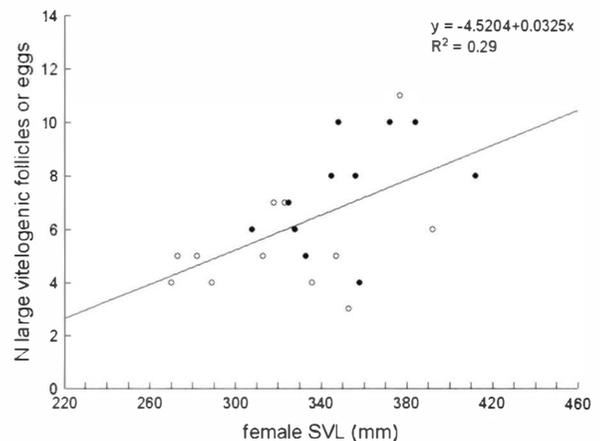


FIG. 4. Relationship between female snout-vent length and clutch size in *Pseudablabes agassizii* from central, south-eastern, and southern Brazil. ($R^2=0.29$, $P=0.003$, $n=39$). Solid circles = eggs; open circles = vitellogenic follicles >10 mm.

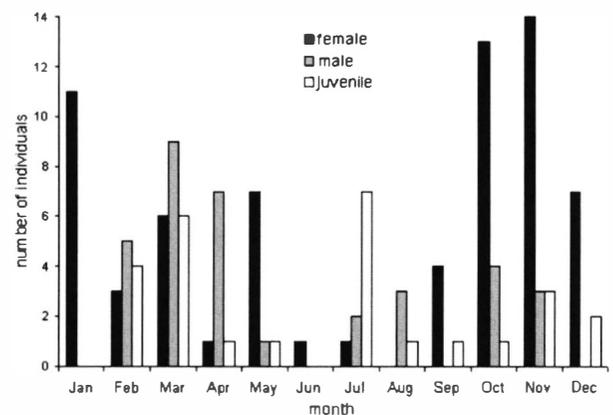


FIG. 5. Seasonal abundance of juveniles, mature males and mature females of *Pseudablabes agassizii*, from collection data of museum specimens. Shaded bars, mature males ($n=34$); solid bars, mature females ($n=68$); open bars, juveniles ($n=27$).

November 2001 laid five eggs (24 November 2001). All eggs of the first female deteriorated. The eggs of the second female averaged 27.10 mm in length (range=24.80-30.75 mm; SD=2.98), 10.02 mm in diameter (range=9.65-10.60 mm; SD=0.35), and weighed a total of 10.3 g. The relative clutch mass was 0.70 (ratio of total clutch weight and mother body weight after egg-laying). Three eggs hatched on 11 February 2002, and the neonates measured 104, 104, and 113 mm SVL. Clutch size ranged from 3 to 11, and was positively and significantly related with maternal SVL ($R^2=0.29$, $P=0.008$; Fig. 4).

SEASONAL ACTIVITY

Adult males and females were registered during all months of the year (Fig. 5), but were significantly more common in the rainy than in the dry season ($\chi^2=23.53$, $P<0.001$, $df=1$). Adult males were more active at the end of the rainy and beginning of the dry season (February - April), whereas adult females show a peak of abundance at the onset of the rainy season (October - January; Fig. 5).

DISCUSSION

BODY SIZE AND SEXUAL DIMORPHISM

P. agassizii is a small snake, adults averaging c. 300 mm SVL. This size is much smaller than that recorded for other species of Philodryadini, which may attain twice this size (see Thomas, 1976; Thomas & Fernandes, 1996; Hartmann, 2001). The small body size of *P. agassizii* is likely related to its food habits, as it feeds exclusively on relatively small prey (see below), whereas other Philodryadini usually prey on relative large vertebrates (cf. Sazima & Puerto, 1993; Hartmann, 2001).

The larger body size in female *P. agassizii* and the value of SSD indicates the absence of male-male combat (cf. Shine, 1994), which remains unrecorded for the neotropical Xenodontinae. Sexual dimorphism was verified in most morphometric parameters analysed, but not in head length. Sexual differences in body size, stoutness, and tail length are a common condition in snakes, and are usually related to reproduction (see King, 1989; Shine, 1994). Head size dimorphism in snakes may be related to intersexual dietary divergence (see Shine & Crews, 1988; Shetty & Shine, 2002). However, *P. agassizii* feeds on small prey and thus sexual differences would be unexpected.

HABITAT USE

P. agassizii is a habitat specialist, at least in south-eastern Brazil, since it was found almost exclusively in only one Cerrado habitat, the shrubby grassland or "campo sujo". The Cerrado domain is composed of distinct vegetational physiognomies (treated herein as habitats), and recent field studies indicate that some snakes select specific habitats (Nogueira, 2001b; Nogueira *et al.*, 2003; Sawaya, 2004). Other Philodryadini, such as *Philodryas olfersii* and *P.*

patagoniensis also show habitat selection (see Hartmann, 2001).

FEEDING AND DIET ACTIVITY

The specialisation of *P. agassizii* on arachnids (over 70% of prey) has been noted previously by Viñas (1985) and Cei (1993). Our data also indicate that insects are a minor dietary item. Lizards are only occasionally eaten and were refused by the captive snake. Thus, vertebrate prey may be regarded as a vestigial trait of *P. agassizii*, present in its ancestral stock. Although phylogenetic analyses are unavailable for Philodryadini, one distinct clade (*P. agassizii* + *Philodryas patagoniensis* + *Philodryas livida*) is supported by three synapomorphies (H. Ferrarezzi, pers. comm.). Most species within the Philodryadini (those in the genera *Philodryas* and *Tropidodryas*) feed on vertebrates (Sazima & Puerto, 1993; Hartmann, 2001). Food habits of *P. livida* are unknown, whereas *P. patagoniensis* prey upon vertebrates (Hartmann, 2001; Marques *et al.*, 2004). However, at least some populations of *P. patagoniensis* from Uruguay usually prey on spiders (Vidal, 2002). Additionally, one specimen of *P. patagoniensis* from southern Brazil consumed one grasshopper and a freshwater shrimp (M. Di-Bernardo, pers. comm.). Thus, the main prey of *Pseudablabe*s (arachnids and other arthropods) occurs in the diet of at least one of its close relatives, and was probably occasionally present in the diet of their ancestor. The specialization of *P. agassizii* on arachnids is thus an autapomorphic trait since such habits are absent in other Philodryadini and xenodontine snakes in general. A comprehensive study on the phylogeny and habits of the Philodryadini would provide useful tools for evaluating our suggestions. Spiders may be regarded as a dangerous prey and few snakes eat them: *P. agassizii* and some sonorine snakes are the only New World snakes that prey primarily upon arachnids (see Greene, 1997). The captive specimen of *P. agassizii* swallowed spiders more frequently abdomen first, perhaps minimizing a spider's defensive response.

Data obtained herein on the captive snake, and observations in the field (Viñas, 1985; present study), indicate that *P. agassizii* is a diurnal snake, which seems characteristic for other Philodryadini as well (Vitt, 1980; Marques *et al.*, 2004). Most prey of *P. agassizii* are active at night and some others (*Bothriurus* scorpions, *Actinopus* and other spiders) are secretive and fossorial during the day (R. Bertani, pers. comm.). Hence, these nocturnal prey are probably taken in their day-time retreats. *Pseudablabe*s *agassizii* probably forages mainly underground, although some diurnal lycosid spiders may be caught on the surface. As this snake catches and holds large prey until its struggles cease, it seems that *P. agassizii* has venom that is toxic to spiders. During the capture and/or manipulation of spiders, the prey can bite the snake, and thus predation on large spiders is rendered possible by the injection of

venom into the prey, and possibly by its own immunity to the prey's venom.

REPRODUCTION AND SEASONAL ABUNDANCE

Five clutches (from seven to 10 eggs) deposited inside ant nests were recorded by Vaz-Ferreira *et al.* (1970) and Vinãs (1985). The data presented here increase this range and indicate that *P. agassizii* produces small clutches when compared to the related *Philodryas patagoniensis* from south-eastern Brazil, which can produce up to 29 eggs (Fowler *et al.*, 1998; FSO pers. obs.). This low fecundity seems to be a function of the small adult size of *P. agassizii*, as the larger species *Philodryas patagoniensis* has larger clutches (FSO, pers. obs.). Moreover, hatchlings of *P. agassizii* are much smaller and lighter than those of *Philodryas patagoniensis* (Fowler *et al.*, 1998). Smaller species producing smaller offspring is a trend found in other neotropical colubrid snakes (e.g. Marques, 1998; Marques & Puerto, 1998; Hartmann *et al.*, 2002; Sawaya 2004). On the other hand, clutch sizes of *P. agassizii* are similar to those of two species of *Philodryas* in north-eastern Brazil studied by Vitt (1980), although the range of SVL of females analysed in that study was larger. Eggs of *P. agassizii* are however much smaller than those of the north-eastern *Philodryas*.

As would be expected for seasonal climates (Cerrado and grasslands in southern Brazil; see Nimer, 1989), seasonality is evident in the activity pattern and reproductive cycle of *P. agassizii*. Moreover, seasonal patterns in its activity and reproduction are similar to those described for other Philodryadini, such as *Philodryas* spp. and *Tropidodryas* spp. (Vitt, 1980; Fowler & Salomão, 1994a; Fowler *et al.*, 1998; Marques *et al.*, 2004; Marques & Sazima, 2004). Variation in rainfall and temperature (especially minimum temperature; Sawaya, 2004) may influence directly the activity of these snakes, but events in reproductive cycles may also influence their activity pattern (see below; Marques *et al.*, 2001).

The female reproductive cycle of *P. agassizii* indicates that egg-laying occurs during the warmest months, allowing clutches to maintain higher temperatures for embryonic development. The peak of female activity in the middle of the rainy season corresponds to the time when they contain vitellogenic follicles or oviductal eggs, when ovulation should also occur. Females probably increase their activity in warmer periods when they may be searching for appropriate thermoregulation or oviposition sites (Marques *et al.*, 2001). The preponderance of adult males from February to April indicates that this is likely to be the mating season. In support of this hypothesis, testes of males attain a larger volume at that time (*cf.* Shine, 1977a). Males might store sperm in deferent ducts and mate later (S. M. Almeida-Santos, pers. comm.), although the absence of a peak in the deferent duct diameter cycle of *P. agassizii* indicates that the produced sperm is used immediately during mating in

February-April. Field records of mating and aggregation in another species of Philodryadini, *Philodryas olfersii*, correspond to the same period (see Fowler *et al.*, 1998, Cechin & Hartmann, 2001). If mating occurs before the dry season and males do not store sperm, then females need to store sperm until the next rainy season when fertilisation would occur.

OVERVIEW

Pseudablabe agassizii is broadly distinct from other Philodryadini in its morphology and ecology. Apparently, its small body size, short tail and reduced dorsal scales (*cf.* Peters & Orejas-Miranda, 1970) reflect adaptations for locomotion in subterranean hollows and crevices, where this species forages. Additionally, ant nests may be suitable for incubation of its eggs. Thus, underground microhabitat in shrubby grassland ("campo sujo" in the Cerrado) seems to be essential for the foraging and egg-laying activity of this snake. The absence of *P. agassizii* in disturbed areas indicates that it is highly susceptible to destruction of natural shrubby grasslands, and thus it is sensitive to habitat alteration and could be an indicator species of environmental quality.

Shrubby grasslands are the dominant landscape at EEI, one of the few remnants of protected Cerrado in south-eastern Brazil (see Sawaya, 2004), and thus warrant special attention in conservation planning. The Cerrado was historically the second largest ecosystem in Brazil, about 2 million km² throughout the country, and has been greatly disturbed in recent decades mainly due to extensive agriculture and cattle ranching (Ratter *et al.*, 1997). But only 20% of this biome remains undisturbed, and only 1.2% is in preserved areas (Mittermeier *et al.*, 1999). Although the Cerrado was recently ranked among the 25 most important terrestrial diversity hotspots (Myers *et al.*, 2000), and is possibly the most threatened tropical savannah in the world (Silva & Bates, 2002), even basic information on its herpetofaunal composition and natural history are mostly unknown (Colli *et al.*, 2002). Our study suggests that *Pseudablabe agassizii* is an important component of the Cerrado snake fauna by virtue its ecological specialisation, and it should be regarded as a threatened species due to rapid destruction of its main habitat.

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APPENDIX 1

SPECIMENS EXAMINED

Pseudablabes agassizii

Central Brazil: IB 4610, 5300, 10502, Campinorte, Goiás (GO); IB 52160, Corumbá de Goiás, GO; CHUNB 3671, 3681, Luziânia, GO; CHUNB 20358, São João da Aliança, GO; CHUNB 3673, 3677, 3679, Brazilândia, Distrito Federal (DF); IB 20544, 20545, 37550, Brasília, DF; CHUNB 3672, 3678, 3680, 3682, 3690, 3720, 3728, 3731, 3782, 14340, 20360, 20363, 23711, 24476, Brasília, DF; IB 68700, Cáceres, Mato Grosso (MT); IB 33797, Ponta Porã, Mato Grosso do Sul (MS); IB 15747, Rio Brilhante, MS; IB 8829, Terenos, MS.

South-eastern Brazil: IB 23378, Aguaí, São Paulo (SP); IB 18666, 31633, Agudos, SP; IB 18757, 18796, 18797, 32873, Altinópolis, SP; IB 135, 4768, 16658, 16659, 43756, Batatais, SP; IB 10319, 32658, Botucatu, SP; IB 5894, Capão da Cruz, SP; IB 10137, Franca, SP; IB 10314 Georgia, SP; IB 52761, 52762, Igarapava, SP; IB 43665, 71328-71337, “Estação Ecológica de Itirapina”, Brotas, SP; ZUEC 2496, 2872-2884, “Estação Ecológica de Itirapina”, Brotas, SP; IB 136, Lençóis Paulista, SP; IB 55727, Morro Agudo, SP; IB 5064, Pedregulho, SP; IB 27762, Pontal, SP; IB 9037, 9707, Restinga, SP; IB 53270, Ribeirão Preto, SP; IB 7196, 7757, 7786, 9099, Rubião Júnior, SP; IB 32060, Araguari, Minas Gerais (MG); IB 46427, 46429, Carbonita, MG; IB 30252, 30256, Cruzília, MG; IB 43661, Diamantina, MG; IB 26784, 29254, 40732, Ibiá, MG; IB 46969, Ituiutaba, MG; IB 12586, 12971, 13555, 13570, 15273, 15511, 15618, Miguel Bournier, MG; IB 12560, Ouro Branco, MG; IB 43071, Parque Nacional Serra da Canastra, MG; IB 37263, Uberaba, MG; IB 28285, 54114, Uberlândia, MG; CHUNB 20364, Unaí, MG.

Southern Brazil: IB 20896, Nova Restinga, Paraná (PR); IB 10247, 10479, 10480, 15947, 15995, Palmeira, PR; IB 10467, Itapitocaí, Rio Grande do Sul, (RS); IB 7853, 7947, 8061, 8062, 8181-8184, 8213, 8323-8325, 8457-8460, Passo Fundo, RS; IB 1652-1659, Pelotas, RS; IB 16026, Restinga Seca, RS; IB 9650, 9876, Rosário do Sul, RS; IB 9867, Santa Maria, RS.

INTERPOPULATION DIFFERENCES IN WATER-SEEKING BEHAVIOUR IN THE GREEN TOAD *BUFO VIRIDIS*

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Many species of terrestrial amphibians can absorb water by pressing a highly vascularised region of the ventral abdominal skin against a moist surface. The degree of dehydration needed in order to evoke this response varies from species to species, suggesting that they make different use of the opportunity to use surface water for hydration. Most of the species that have been studied are each confined to a fairly narrow ecological niche, and the differences in their responses make sense in the light of their natural history. Consequently, it was of interest to compare populations of a single species living in different regions to see whether differences in the response to the presence of moisture are characteristics of the species or are related to local conditions. The steep north-south gradient of increasing aridity in Israel and the presence of populations of the toad *Bufo viridis* throughout the country provided an opportunity to make such a comparison. The frequency of this water seeking response (WR) in fully hydrated toads and the sensitivity of WR to raised plasma osmolality were determined in laboratory-acclimated samples collected from three sites in Israel, one from north-west Italy and one from Kyrgyzstan. These sites differed in altitude and climatic characteristics (mean annual temperature and total annual rainfall). A negative correlation was found between the frequency of WR and the annual rainfall at the sites where samples were collected, suggesting that WR is not a fixed characteristic of this species. The observed differences between populations are discussed with reference to evolved adaptations to aridity and the past evolutionary radiations of the species.

Key words: amphibian, adaptation to aridity, green toads, opportunistic hydration

INTRODUCTION

Most amphibians do not drink; that is, they do not swallow water. Rather, they absorb water through their skin (Bentley & Yorio, 1979). Yet, outside the breeding season, terrestrial amphibians immerse themselves in water for only relatively short periods; frequently they satisfy their water requirements by pressing a highly vascularised region of the ventral abdominal skin, the "seat patch" (Hillyard *et al.*, 1998), against a moist surface. This behaviour, described in dehydrated anurans by Stille (1958), can be observed in some species in laboratory conditions, even in the presence of standing water (Tran *et al.*, 1992).

In a long series of experiments, Hillyard and co-workers investigated factors influencing the expression of this behaviour (reviewed in Hillyard *et al.*, 1998). They found, *inter alia*, large interspecific variations in the degree of dehydration needed to evoke a consistent water absorption response, and noted that the differences between species make sense in the light of their natural history and of the environment in which they are found. The implication of their observation is that there may be evolved differences in the toads' use of opportunistic hydration. Since each of the species they studied is confined to a fairly narrow ecological niche, it became of interest to compare the responses of toads taken from different populations of a single species that lives in a variety of climatic conditions.

Such a comparison could help to determine whether the conditions required to elicit the toads' water absorption response are a fixed and constant characteristic of the species, or vary from population to population in a way that is correlated either with their geographical distribution or with their type of habitat.

The green toad, *Bufo viridis*, provides an opportunity for this type of comparison. It is widespread in Eurasia, extending from Mongolia westwards to the Rhine, and from southern Sweden to the Iberian peninsula and across to North Africa. Distant populations inhabit widely different climatic conditions. In Israel, situated between Eurasia and Africa at the eastern edge of the Mediterranean Sea, the toad approaches the southern limit of its distribution. The "crossroads" geographical position of Israel has endowed it with a diverse fauna, which includes immigrants from both north and south (Nevo, 1988). It also has a steep north-south gradient of increasing aridity, in which a mesic Mediterranean climate gives way to Irano-Turanian steppe and then to Saharo-Arabian desert in less than 400 km. Populations of *B. viridis* are widespread throughout the mesic and steppic regions, and have spread into the desert, following the line of irrigation as agriculture has been developed (Werner, 1988). There are also small isolated, possibly relict, populations at a number of oases bordering the desert. The region, therefore, provides a good opportunity to compare populations of the toad living in different habitats within a small geographical area, and to see the extent to which they differ from each other and from those from more distant regions.

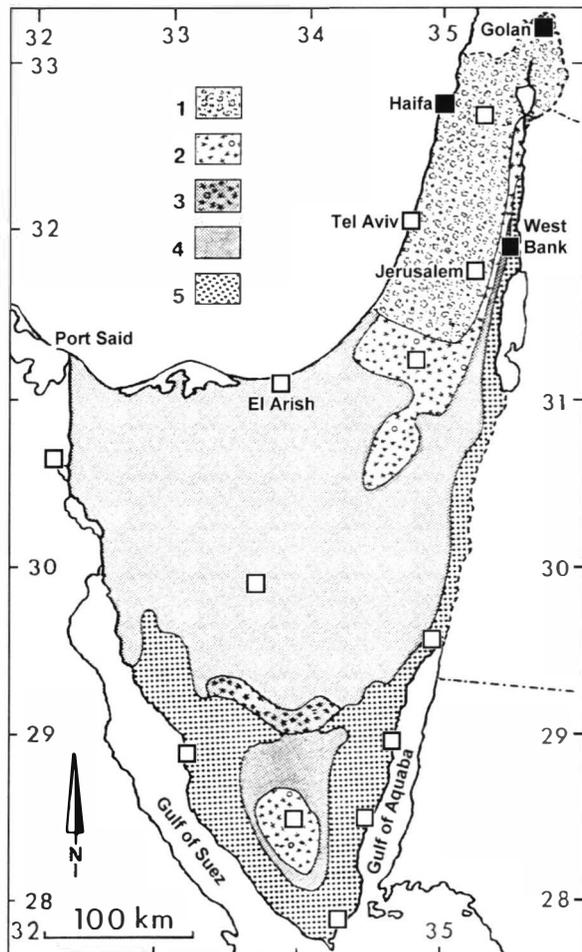


FIG. 1. Biogeographical regions of Israel and Sinai, based on Werner (1988). 1. Mediterranean; 2. Irano-Turanian; 3. Irano-Turanian/Saharo-Arabian transition; 4. Saharo-Arabian; 5. Sudanian penetration zone. Filled squares: collection sites in the present study.

In *B. viridis*, and probably in other species of terrestrial amphibians, the water absorption response consists of two steps. The first, water seeking with postural ad-

justment when moisture is detected (WR), is followed by the second, water uptake across the skin of the "seat patch" (Hoffman & Katz, 1999). In this study, I focused on the first step and compared the frequency of the response (WR) and its sensitivity to artificially raised plasma osmolality in samples of toads collected from three sites in Israel, from one in north-west Italy and one in north Kyrgyzstan. Since aldosterone, which is released under the influence of the hormone angiotensin, is known to augment the water seeking response in amphibians (Hillyard *et al.*, 1998; Hoffman & Katz, 1999), I also studied the effects of this steroid on the behaviour of toads from the different sites.

MATERIALS AND METHODS

Details of the samples of toads collected from the West Bank of the Jordan, Haifa Bay and Golan Heights areas of Israel, Vigone in north-west Italy and Kokjar in Kyrgyzstan are given in Table 1; those from the West Bank and Haifa were part of a study reported earlier by Hoffman & Katz (1999). Climatic data within Israel were obtained from meteorological stations, each within 25 km and at the same altitude as the specific collection sites. The microclimate within the West Bank wadi does not conform to the general pattern in Israel of an increase in aridity from north to south (Fig. 1), but surrounding areas at higher altitudes have a much lower annual rainfall (Meteorological Services Reports, 2000; unpublished Meteorological Services data, personal communication). Meteorological data for north-west Italy and for Kyrgyzstan are those given in Castellano & Giacoma (1998) and Castellano *et al.* (1999) respectively, and are based on the records for meteorological stations in Walter and Leith's Klimadiagramm-Weltatlas (1967).

Toads were collected at dusk, when they emerge from their shallow burrows, before they had an opportunity to reach a water source. They were acclimated to labo-

TABLE 1. Characteristics of the toads and climate conditions at the collection sites.

	West Bank	Haifa Bay	Golan	Vigone	Kokjar
Sample Size	10	10	12	9	11
Mean Standard toad weight (g)	38.3±9.97	34.9±3.80	36.5±13.33	23.3±1.71	48.6±10.44
Period of observations	Dec 1997 - May 1998	Mar 1998 - May 1998	Jan 2000 - Mar 2000	May 1998 - Jul 1998	May 1998 - Jul 1998
Altitude (m asl)	250	50	940	300	1350
Annual mean temperature (°C)	17.5	20.0	15.3	12.2	9.2
Total annual rainfall (mm)	450	399	800	680	375

ratory conditions for at least two weeks before testing. Throughout the acclimation and experimental periods they were maintained at room temperature ($20\pm 2^\circ\text{C}$) and were provided with a pool and dry area. They were fed mealworms, *ad lib.*, once a week, but did not receive food during the 48 hr preceding an experimental trial.

The observations were made over a period of three years, and to test whether there were changes in the populations sampled or laboratory conditions during this period, WR frequency in untreated toads was replicated in additional samples taken from two of the sites: seven additional toads, mean standard weight (m.s.w.) 25.3 ± 4.62 g, from Vigone were the subjects in September 1999, and four from the West Bank (m.s.w. 37.7 ± 5.20 g) were observed in June 2000.

The experimental protocol was as described in Hoffman & Katz (1999), but note that that paper gives incorrect values for the quantities of NaCl injected. In brief, toads taken from the "home" dry area were catheterised in order to empty the urinary bladder, weighed, and introduced individually into observation chambers. Two filter paper discs, one dry and the other saturated with distilled water, were positioned on the floor of each container, and the animal was placed between them. Behavioural responses were monitored for up to 3 hr by observing the animals through angled mirrors beneath the chambers, the walls of which were covered to prevent the animals seeing their observers. WR was deemed to have occurred if the animal flattened itself on the wet filter paper and had gained weight after this response. Previous work has shown that neither catheterization, nor an i.p. injection *per se* (with 0.8% NaCl) affected WR frequency (Hoffman & Katz, 1999).

In each group, WR frequency was determined under three conditions: (1) untreated (control); (2) with increased plasma osmolality – the concentrating effect of dehydration to 7% weight loss was simulated by intraperitoneal (i.p.) injection of a NaCl solution (30 $\mu\text{mole}/100$ g toad); see Hoffman & Katz (1999); and (3) with increased plasma osmolality and aldosterone – 15 min after the above salt treatment, aldosterone (Sigma: Rehovot, Israel) was given by i.p. injection of 250 $\mu\text{g}/100$ g toad. In those groups that were very sensitive to the raised plasma osmolality (>80% WR response), the salt concentration was reduced by 50% to 15 $\mu\text{mole}/100$ g toad to allow a margin in which to test for any additional effect produced by aldosterone.

The toads were weighed and placed in the observation chambers immediately after completion of treatments 2 or 3. All observations were made between 0700 and 1200 hrs.

The dehydration threshold, i.e., the lowest level of dehydration at which all animals of a group exhibit WR on all occasions, was determined for toads from the Golan using the method of Hoffman & Katz (1999). Six bladder-emptied toads were subjected to rapid dehydration for 2-3 hrs in small wire cages in an airstream generated by a fan. At intervals, each was weighed, % weight loss was calculated, and WR behaviour was ob-

served until a level was reached at which all animals showed the response. The procedure was repeated three times; the least % weight loss at which all animals showed WR was taken to be the dehydration threshold. The same toads were treated with aldosterone and three further trials were carried out to establish the threshold after this treatment.

Because of the legal and ethical constraints on collecting this species in Israel and on importing them, the numbers of toads in the samples from all regions were necessarily small. Consequently, between 4 and 11 observations were made on each toad over a period of approximately three months, with a minimum of two days rest between handling. Possible systematic changes in the toads' behaviour over time were examined statistically using a sign test to compare the number of positive WRs made by each toad in the first half of the observations with that for the last half. To see whether there was heterogeneity in the responses of individual toads from the same site, the observed numbers of toads showing different proportions of positive responses were compared with the numbers predicted from a binomial distribution based on the average for the group as a whole. The *G*-test of independence was used to assess whether the proportion of positive responses differed between groups from different collection sites or the same site at different times. The effects of the three climatic factors on the response were analysed by stepwise regression (SAS: GLM procedure). A one-tailed paired *t*-test was used to compare the percentage of positive water responses of toads from the five sites before and after salt injection.

RESULTS

Levels of WR in untreated animals from populations from Vigone and the West Bank site remained stable over time (Table 2). Times of collection and study were therefore excluded from all analyses. There was also no evidence for any group of systematic changes in WR over time spent in the laboratory: when the groups were pooled, 27 of the 52 toads observed showed no difference in the number of responses in the first and last half of the observations, 8 responded more in the first half, and 17 more in the second half. The difference is not significant ($0.1 > P > 0.05$). Table 3 shows there is also no evidence of heterogeneity between toads from the same site: the observed numbers of toads showing different proportions of positive responses are very close indeed to those predicted from a binomial distribution based on the average for the group as a whole. It was therefore assumed that repeated observations on the same toad were independent of each other, and for statistical analyses observations on all toads within a group have been pooled and treated as a single set of observations.

Table 4 shows (1) the spontaneous expression of WR in fully hydrated toads differed between groups ($G_{141}=47.8, P<0.005$), i.e. it was not independent of site. It was very low (6-8%) in the groups from Golan (northern Israel) and Vigone (NW Italy) compared with those

TABLE 2. Water responses of toads collected and tested at different times.

Site	Year	Water response	No response	%WR
Vigone	1998 (May-July)	5	74	6
	1999 (Sept-Oct)	2	34	6
West Bank	1998 (Dec-May)	12	38	24
	2000 (June-July)	5	19	21

TABLE 3. Number of responses made by each toad compared with the number expected on the basis of the binomial distribution. (O is the observed number of toads making each number of responses; E is the number expected from a binomial distribution based on the mean number of responses of all toads from the site.)

Site	West Bank		Haifa Bay		Golan		Vigone		Kokjar	
Number of toads	10		10		12		9		11	
Observations per toad	5		4		8		11		6	
Number of responses	O	E	O	E	O	E	O	E	O	E
0	3	2.5	0	1.4	6	6.2	5	4.6	0	0.3
1	4	4.0	4	3.5	4	4.3	3	3.2	2	1.6
2	2	2.5	5	3.4	2	1.3	1	1.0	3	3.1
3	0	0.8	1	1.4	0	0.2	0	0.2	5	3.3
4	1	0.1	0	0.2	0	0	0	0	1	1.9
>4	0	0	-	-	0	0	0	0	0	0.6

from the other three sites. (2) Increasing the plasma osmolality by salt injection increased WR frequency in all groups ($t_{141} = 8.29, P < 0.005$). (3) In the two groups with low levels of WR in the untreated state, treatment with salt and aldosterone did not significantly increase WR frequency above that of salt alone, although large increases were seen in each of the other three groups.

There was a significant negative correlation ($r = -0.923, P < 0.05$) between % WR and total annual rainfall in the areas from which the groups were collected. Neither altitude, mean temperature, nor the interactions between the three climatic factors exert any further significant influence ($P > 0.05$).

The dehydration threshold of toads from the Golan was found to be marginally less than an 8% loss of their

standard weight, and treatment with aldosterone had no effect on this threshold. This compares to a threshold of 14% loss, reduced to 7% by aldosterone, in toads from the West Bank (Hoffman & Katz, 1999).

DISCUSSION

The results show that there were differences between the spontaneous water seeking response of untreated, fully hydrated *B. viridis* that had been collected from different sites and acclimated to laboratory conditions.

The phrase "fully hydrated" has not been used consistently in the literature, but here, as in a previous study (Hoffman & Katz, 1999), I use it in the sense that the toads had no apparent drive to enter water, choosing to

TABLE 4. Percentage water response in toads, *Bufo viridis* (number of individual observations in parentheses).

Treatment	West Bank	Haifa Bay	Golan	Vigone	Kokjar
none	24 (50)	39 (44)	8 (96)	6 (79)	44 (62)
NaCl	60 (20)	90 (20)	41 (64)	30 (71)	85 (20)
NaCl + aldosterone	95 (20)	-	41 (56)	39 (36)	-
NaCl (half strength)	40 (10)	70 (20)	-	-	50 (24)
NaCl (half strength) + aldosterone	-	100 (20)	-	-	93 (28)

sit in a dry area when a pool was freely available. Of course, this does not mean that all had the same total water content and urine volume, only that behaviourally they showed no signs of dehydration.

In captivity, even when housed in a group, these toads act individually regarding when they enter a pool and how long they spend in water, only entering water together after feeding or handling. So, if left undisturbed, it can be assumed that animals in the dry area of the terrarium have been out of water for different periods. In still air, in the controlled conditions of the laboratory, the amount of evaporative water loss (EWL), and hence the plasma osmolality of an individual, will depend on when it was last in water, so a group will be made up of animals with different plasma osmotic concentrations. Since raised plasma osmolality stimulates the water response (Hoffman & Katz, 1999), it follows that the level of spontaneous WR in a group probably reflects the mean period between immersions. On this basis, the results in Table 4 suggest that of any four West Bank toads taken at random from the dry area of the terrarium, one is near the level of plasma osmolality that stimulates WR: in contrast, only rarely is a toad from the Golan in this state. Stated another way, the data suggest that Golan toads enter water more frequently. The differences between the groups are not a direct outcome of weight-related differences in EWL, since toads from the Golan and from NW Italy (Vigone) have similar % WR (Table 4) even though the animals from the Golan are, on average, more than 50% heavier than those from NW Italy (Table 1). The WR is, however, correlated with the total annual rainfall in the areas from which the toads were collected: those from more arid areas showed a more frequent response.

There are several possible interpretations of the observed differences. One is that they are chance outcomes of genetic changes that occurred in the populations during periods when they were isolated. Nevo (1988) claimed that *B. viridis* has a continuous population structure in Israel, but the findings of Maxson (1981), who included populations of *B. viridis* from six sites in Israel in a wider survey of phylogenetic relationships of Eurasian *Bufo*, do not support this. Using an immunological technique to measure differences in the amino acid composition of albumin, she estimated that "a mesic population from northern Israel near the Lebanese border" (i.e. Golan) had been isolated from the others for 0.6-3 million years. The marked difference in WR and sensitivity to aldosterone found in the present study could therefore be the result of divergent genetic changes that occurred while toads in the Golan were isolated from those now found further south.

The present indigenous fauna of Israel includes immigrants from both north and south (Nevo, 1988), so today's populations of *B. viridis* could be the descendants of two independent migrations. According to Savage (1973), the species radiated from Asia during the Pleistocene (Great Ice Age) and followed two major migratory routes. One was westwards across Europe to

the Iberian peninsular, and the other went south-west, through what is now Turkey, with branches leading to the Indian subcontinent and the East Mediterranean coastal strip. Later, during the Holocene (Recent) epoch, the animals reached NW Africa via the European route. Today, populations of *B. viridis* are found along the North African Mediterranean coastal strip, including Morocco in the west and Egypt in the east. So, at least theoretically, toads could have reached Israel either via the long route, westwards across Europe and then eastwards along the North African coast, or by the shorter route from the north-east, or by both routes. However, a phylogenetic tree based on the analysis of nuclear DNA suggests they came by the shorter route (E. Balletto, personal communication). Balletto found that Asiatic toads of the *viridis* group (from 10 sites) and mid-European populations of *B. viridis* (from eight sites) formed two distinct clusters, which diverged a long time ago. On the same basis, the genetic distance between toads from the European sites and those from three sites in Israel (which included the Golan and West Bank) was estimated to be 3-4 times greater than the distance from the Asiatic populations. If, as Nei (1972) argued, genetic distance is related to the geographical distance of a migratory route, Balletto's data suggest that *B. viridis* entered the Levant only from the north. It therefore seems unlikely that the difference in WR between the Golan and the other two Israeli populations is a result of two independent migrations, one from Asia and one via Europe and N. Africa.

An alternative explanation is that the difference between populations in Israel is the result of evolutionary adaptations that occurred as *B. viridis* spread southwards into xeric habitats. As they moved into more arid regions, any behavioural and physiological adjustments that reduced the need for frequent access to water would have been an advantage. There would have been strong selection for the ability to tolerate higher plasma osmolality, which would allow longer intervals between entering water, and also, because of the favourable osmotic gradient across the skin, increase the ability to absorb soil-bound water through the water response. In the laboratory, a high WR was found only in toads from populations living in xeric habitats. Two geographically distant groups, those from Golan and Vigone, which both live in mesic conditions, showed a much lower response. However, the results following salt injection show that raised plasma osmolality, a state that toads living in mesic habitats probably rarely experience, did increase their water seeking response, indicating that they have the potential to adapt to more arid conditions.

The observation that all toads from the Golan population showed WR when they had lost only 8% of their weight through dehydration, whereas those from populations in xeric regions do not do so until they have lost 14% of their weight (Hoffman & Katz, 1999) is further evidence of the greater tolerance to raised plasma osmolality of toads from arid environments. *B. viridis* can survive more than 30% weight loss (almost entirely

due to lost water), so there is still an enormous safety margin, but the sensitivity to water loss seems to be partially suppressed in toads inhabiting the more arid regions. However, it can be reactivated by aldosterone: the present study shows that this steroid has no effect on the dehydration threshold of Golan toads, which remained at just under 8%, whereas in Hoffman & Katz's (1999) study of West Bank toads it reduced the dehydration threshold from 14% to 7%. If cues associated with the onset of the often unpredictable and irregular rainfall of arid environments are the stimulus for hormonal release, this could add flexibility to the behavioural response of toads in xeric habitats.

Travelling south from the Israel-Lebanon border today, the climate becomes increasingly arid, but there are no physical obstacles great enough to deter toads from migrating between the Golan area and regions further south. However, over the last 1.8 million years there have been great changes in paleogeography and environmental conditions in the region (Horowitz, 1988), and volcanic activity persisted on the Golan Plateau and neighbouring areas at least into the early Pleistocene (Garfunkel, 1988). Any one or more of these climatic or geological events could have temporarily cut off the northerly populations from those which had been established further south, and initiated genetic divergence through drift, the occurrence of different mutations in the populations, or selection for adaptations to dissimilar ecological conditions (Schluter, 2001). If the differences in the WR found in this study are the result of genetic divergence, they must be maintained either by strong selection, or by some degree of reproductive isolation that reduces gene flow between populations. Maxson's (1981) survey shows that a population only about 30 km south of the Golan (Dalton) has distinctly different albumin from that of the Golan toads, although DNA analyses have revealed no large differences between toads from the Golan and other Israeli locations. However, pre-zygotic isolation between populations can result from very few genetic differences (Coyne, 1992). An important pre-mating isolating mechanism in some amphibians is the advertisement call of the male, and Castellano *et al.* (2002) have shown that call patterns in *B. viridis* can differ more than the genetic distances between populations would predict. A comparative study of advertisement calls in the Golan and other regions would therefore be interesting, since selection in acoustically different environments could have led to divergence. Selection for a shift in the time of breeding and/or a change in the water temperature at which mating takes place could also lead to a mating barrier between populations. *B. viridis* in Sardinia breeds in water below the temperature at which those on the mainland are able to do so (Castellano & Giacoma, 1998), and the situation in the Golan may be similar, since at the turn of the year early rains are supplemented by the melting snows of Mt. Hermon, resulting in cool, fresh-water ponds and rivulets. At present we can only guess

at why the Golan population remains different from those in other regions in Israel, but it is reasonable to assume that any physical barrier that persisted long enough for genetic divergence in the sensitivity to water loss and use of WR would also have allowed divergence in potential isolating mechanisms.

There is a further possible explanation of the differences in WR frequency in toads from different populations: use of the response may be determined by the conditions in which the toads developed rather than by genetic differences. In the present study all toads were acclimated to laboratory conditions before testing, and their behaviour did not change over the months spent in the laboratory, but their behavioural phenotype may have been permanently set by the conditions they experienced in the pre-adult stages. If so, the differences in WR between samples from different sites reflect the behavioural plasticity of the species, rather than genetic divergence between populations. The similarity of the response of geographically distant populations inhabiting areas with similar annual rainfall is consistent with this interpretation, as rainfall determines the availability of the moist areas that are essential for the survival of vulnerable post-metamorphs, although the microclimate of the places where each individual develops will be the ultimate determining factor. If the frequency of WR is a response to the conditions experienced during ontogeny, then local variations in rainfall and other conditions would be expected to lead to differences between samples taken in different years. None were found in the present study, but the samples were small; it would require sampling over several years coupled with a detailed analysis of the microclimate at the sampling sites to determine the influence of juvenile conditions on the adult WR.

Whatever the explanation of the differences, this study of WR in populations of the same species living in very different climatic conditions has indicated a relationship between behaviour and habitat. The adaptation to aridity is apparent only in the water-seeking response, which is the first of the two steps involved in rehydration. In separate experiments (not reported here), no difference was found in the rate of water uptake in toads from the West Bank and the Golan, or in the effect of oxytocin on that rate. Reshetnikov (1998) has found that *B. viridis* is able to detect vapour pressure gradients, and judging from the observations reported here, in which the toads that exhibited WR rarely explored the observation chamber but immediately moved towards the wet disc, they can sense the presence of moisture from a distance of more than 30 cm.

Optimal exploitation of all water sources is vital to amphibians in xeric habitats, and adaptations such as an increased use of WR probably contributed to their ability to survive in these conditions. Whether or not the adaptation is a result of genetic changes or reflects developmental plasticity that is present in all populations of *B. viridis* remains to be determined. Ideally, newly-

fertilized egg strings from different populations would be reared in the laboratory in a variety of conditions, but technically this would be difficult and probably is not feasible. Sampling more toad populations from sites throughout Israel and neighbouring countries over several years, accompanied by close monitoring of the local climatic conditions, would be an alternative approach. There is also a clear need for a more detailed phylogeographic analysis of the toads in these regions using mitochondrial and satellite DNA, which might indicate whether an investigation of possible isolating mechanisms would be worthwhile. There is certainly a lot more work to be done, but the results presented here suggest that studies of *B. viridis* could yield valuable insights into how terrestrial amphibians have been able to move into arid regions.

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PIVOTAL TEMPERATURE FOR GREEN SEA TURTLES, *CHELONIA MYDAS*, NESTING IN SURINAME

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Sexual differentiation of green sea turtles is directed by incubation temperature. The constant incubation temperature that produces both sexes is known as the pivotal temperature, with warmer temperature producing more or all females and cooler temperatures producing more or all males. Here we present data on a laboratory experiment designed to evaluate the pivotal temperature of green turtles from Suriname. The best estimates for the pivotal temperature were 29.4 or 29.5 °C. These values are similar to a previous estimate of pivotal temperature from this green turtle population. When both datasets are combined, the pivotal temperature is estimated to be 29.2 or 29.3 °C. These values are within the range of limited information available from other green turtle populations. Nevertheless, more data from pivotal temperature experiments are needed for a greater understanding of how incubation temperature impacts local nesting populations.

Key words: egg, chelonia, incubation, pivotal temperature, sex ratio, TSD

INTRODUCTION

In many different reptile species, the direction of sexual differentiation is determined by temperature (Shine, 1999). This phenomenon is known as temperature dependent sex determination (TSD) and is often characterized by a pivotal temperature, which is the constant temperature producing equal numbers of each sex, and a transitional range of temperatures or TRT, which consists of those incubation temperatures that produce both sexes (Mrosovsky & Pieau, 1991). The TRT usually spans only a few degrees Celsius, and for incubation temperatures above and below this, all offspring produced are of one sex. All sea turtle species exhibit TSD (Wibbels, 2003). Unfortunately, it is not easy to obtain specific information on pivotal temperatures of sea turtle populations. The expense of precise incubators, the complications of collecting and transporting sea turtle eggs from nesting beaches to laboratory incubators, the labour intensive histology required to classify sex definitively, and the logistical challenge of obtaining permits are some of the disincentives to undertaking a sea turtle pivotal temperature research project. Indeed, despite the discovery of TSD in sea turtles 25 years ago (Yntema & Mrosovsky, 1979), relatively few direct data have been published on pivotal temperatures or TRT for these species (Wibbels, 2003).

Nevertheless, there is a need for more information on pivotal temperatures in sea turtles for a variety of reasons. For instance, there is no validated non-lethal method for classifying the sex of sea turtle hatchlings. Therefore, the sex ratios of offspring produced at a specific sea turtle nesting beach are often derived indirectly by comparing nest temperatures in the field with pivotal temperatures from the laboratory (Baptistotte *et al.*,

1999; Binckley *et al.*, 1998; Broderick *et al.*, 2000; Estes *et al.*, in press; Godley *et al.*, 2001; Godley *et al.*, 2002; Hanson *et al.*, 1998; Mrosovsky *et al.*, 1992; Öz *et al.*, 2004). It is unclear how accurate these estimates are, particularly when nest temperatures are close to pivotal temperatures. Perhaps when nest temperatures are outside of the TRT (e.g. Hanson *et al.*, 1998), the accuracy of estimated sex ratios increases, although few direct validations of the sex ratio estimates of sea turtle hatchling production have been presented to date.

A further problem with comparing nest temperatures with laboratory pivotal temperatures is that TSD data do not exist for all nesting populations of sea turtles. In these cases, researchers often use pivotal temperature data derived from a population elsewhere in the world (e.g. Standora & Spotila, 1985; Baptistotte *et al.*, 1999; Estes *et al.*, in press). Even if data on pivotal temperature are available for the relevant population, they may be based on only one or two clutches of eggs. This may not be a serious drawback if pivotal temperature in sea turtles is a conservative character, as has been suggested (Mrosovsky, 1994). However, so far few data have been available to adequately assess this point.

For these reasons, we present data on pivotal temperature from green sea turtles (*Chelonia mydas*) nesting in Suriname. This nesting population had previously been the focus of several studies concerning offspring sex ratios (Mrosovsky, 1982; Mrosovsky *et al.*, 1984; Godfrey *et al.*, 1996). The present study will increase the database and our knowledge of TSD in sea turtles.

MATERIALS AND METHODS

EGG COLLECTION AND TRANSPORT

The green turtle eggs came from Matapica beach in Suriname (5 59.10N, 54 56.14W). On the night of 8-9 May 1995 eggs were obtained from two separate clutches soon after being laid. At 22.30 hrs local time,

90 eggs were taken from one clutch (designated Clutch N), and under supervision of the Surinam conservation officer, the remaining eggs were placed back in the original nest hole. At 00.30 hrs local time, all 108 eggs were taken from another clutch (designated Clutch O). Eggs from each clutch were carefully placed in a Styrofoam box.

The egg boxes were transported on foot for approximately 6 km, then by a small boat to Paramaribo, and finally by plane to Toronto, Canada. Whenever possible, the eggs were kept in air-conditioned rooms or enclosures to provide low temperature during transport (Harry & Limpus, 1989). The eggs arrived in Toronto at 00.30 hrs local time on the night of 9-10 May, and they were unpacked and placed in the incubators by 03.00 hrs local time (i.e. <30 hours after collection from the beach).

LABORATORY INCUBATION

Each egg was randomly assigned a number and placed singly in a 500 ml covered plastic container that contained 60 ml of deionized water, an indented piece of sponge and moistened vermiculite (further details in Mrosovsky, 1988). Up to 16 eggs (in two layers) were placed on either two or three shelves of five incubators set at different temperatures (shelf temperatures given in Table 1). Sixty five ml of deionized water were added to each egg container on days 17 and 41 of incubation.

A glass mercury thermometer (with 0.1 °C scale) encased in a tube of glycerol was placed on each shelf with the thermosensitive bulb roughly in the middle of the shelf. The thermometers were read once daily with an effort to minimize temperature changes in the incubators. To assess evaporative cooling, on day 30 we inserted a needle-thermist probe into one egg to compare its core temperature to that of an adjacent vial of glycerol. After 24 hrs allowed for equilibration, four readings were taken over two days. The egg core was cooler on average than the glycerol by 0.25 °C. Therefore, a correction factor of 0.25°C was subtracted from all incubator temperature readings to account for evaporative cooling.

After day 45 of incubation, the eggs were checked twice daily for signs of hatching. An egg was considered hatched if the head and at least one flipper of the hatchling were outside of the shell (Godfrey & Mrosovsky, 1997). Incubation duration in the laboratory was calculated as the number of days between laying and hatching. When a hatched egg was found, it was removed from the incubator, the hatchling was quickly killed and the gonads excised and placed in buffered 0.9 % formalin. Following fixation in the formalin for at least seven days, the gonads were prepared as outlined in (Mrosovsky *et al.*, 1984). Briefly, one gonad from each hatchling was cut in half transversely, and embedded in paraffin wax. Serial sections (10 µm thick) were taken from the cut end of the gonad and mounted on slides.

SEXING

The sections were stained with Harris' haematoxylin and periodic-acid-Schiff reagent (PAS), and examined under a light microscope. Male gonads were characterized by a thin smooth cortex and presence of immature seminiferous tubules; female gonads were characterized by a PAS-positive tunica albuginea between the cortex, which was thickened and infolded, and the medulla, which lacked tubules (for details, see Yntema & Mrosovsky, 1980; Miller & Limpus, 1981). Rarely, some gonads exhibited characteristics of both testes and ovaries. They were labeled intersexes and treated as non-females in the calculations of sex ratio (Pieau *et al.*, 1994). All gonads of embryos that died at a late stage were examined for sex, although usually gonadal tissue from these embryos had degenerated beyond recognition.

Two experienced researchers independently evaluated sections from each gonad. In the rare cases when identification of sex by the two disagreed, the two researchers re-examined the tissue sections together. If consensus could not be reached (this occurred only in a few cases when the samples in question had come from embryos that had died prior to pipping and the tissue had deteriorated), no sex was assigned and those samples were excluded from further analysis.

DATA ANALYSIS

The pivotal temperature was calculated according to three different methods. The first was the simple method, based on taking the data point that falls on the 50% female sex ratio level, or, if such a point is not available, by fitting a straight line to join the two data points that fall closest to the 50% female sex ratio level, and taking pivotal temperature as the point where the line intersected the 50% sex ratio level (Mrosovsky & Pieau, 1991). The second was to use maximum likelihood analysis (TSD software ver. 3.2.2, available at <http://www.ese.u-psud.fr/epc/conservation/TSD/index.html>) to place a best-fit sigmoidal curve to the data (Girondot, 1999). The third was to fit a sigmoidal

TABLE 1. Constant incubation temperatures (taking into account -0.25 °C correction factor for evaporative cooling) for green turtle eggs from Suriname. ^aincludes two unhatched embryos that were able to be classified by sex; ^bincludes one intersex (treated as not female)

Temperature ± range °C	Eggs set (N,O clutch)	Eggs hatched (N,O clutch)	Sex ratio (% female)
27.6±0.3	24,24	1,17	0.0 ^a
28.2±0.3	14,16	1,9	8.3 ^a
28.4±0.3	12,16	1,10	18.2
28.7±0.4	16,16	2,11	35.7 ^b
29.4±0.4	16,16	2,11	46.7 ^a
30.0±0.5	4,10	0,4	50.0
30.6±0.4	4,10	0,5	100.0

curve to the data using Prism4 (GraphPad, San Diego, Calif.). To determine the pivotal incubation duration (the incubation period that produced equal numbers of both sexes), we also fitted a sigmoidal curve to data on incubation period of eggs vs. sex ratio, using Prism4. For the curve fitting using Prism4, the pivotal results were nearly identical (<0.1 difference) when we used the raw % female data or arcsin transformed data (Zar, 1999).

RESULTS

Only seven out of 90 eggs from Clutch N hatched (7.8% success rate), while Clutch O produced 67 hatchlings (62.0% success rate; Table 1). The overall hatch rates were significantly different between clutches N and O ($\chi^2=59.45$, $df=1$, $P<0.0001$). Most of the unhatched eggs from Clutch N contained embryos that had died early during development, implying the eggs in the clutch were fertilized. Of the unhatched eggs from clutch N, two were successfully classified by sex; from clutch O, four unhatched eggs were accurately classified

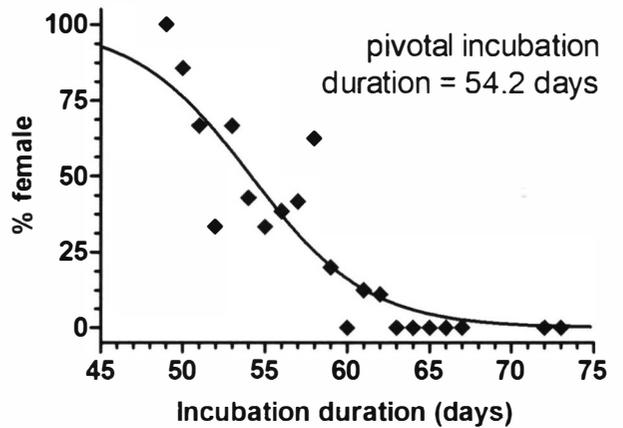


FIG. 2. Relationship between days to hatching and sex ratio in laboratory-incubated green turtle eggs. Data were smoothed using a 2-day running window mean. The sigmoidal curve was fitted to data using Prism4 software.

by sex. One hatchling from clutch O was rated as an intersex. There was no significant correlation between incubation temperature and hatching success of either clutch or both combined (Spearman rank correlation, $P>0.05$).

Some of the different shelves in the incubators had similar incubation temperatures during the experiment. In these cases, the results were pooled (Table 1). Based on the simple method of Mrosovsky & Pieau (1991), the pivotal temperature was 30.0 °C (Fig. 1A). Using Girondot's (1999) method of fitting a sigmoidal curve using maximum likelihood analysis, we derived a pivotal temperature of 29.4±0.1SE °C. When a sigmoidal curve was fit to the data using Prism4 software, the pivotal temperature was estimated to be 29.5±0.1SE °C (Fig. 1A).

Data on incubation period (time to hatching) and corresponding sex ratio of eggs that hatched were smoothed using a 2-day running window mean, and had a sigmoidal curve fitted to them using Prism4 software. The laboratory pivotal incubation duration was 54.2±0.7SE days (Fig. 2).

DISCUSSION

Methods for calculating pivotal temperatures of sea turtles have varied across studies (e.g. Limpus *et al.*, 1985; Mrosovsky, 1988; Chevalier *et al.*, 1999). Indeed, a derived pivotal temperature value from a single dataset can vary 1 °C or more, depending on what procedure is employed (Mrosovsky & Pieau, 1991). In the present case, the small discrepancies in pivotal temperature values among the analyses used were nearly all smaller than the range of constant temperatures recorded during incubation (Table 1). In the 1995 dataset, four eggs incubated at 30 °C produced exactly 50% females. For a point on the steep portion of the sex ratio curve, a sample size of four eggs is too small to be reliable for some methods. If data for that point were excluded, the pivotal temperature would be 29.5 °C, based on the simple method (Fig 1A). This highlights a potential weakness

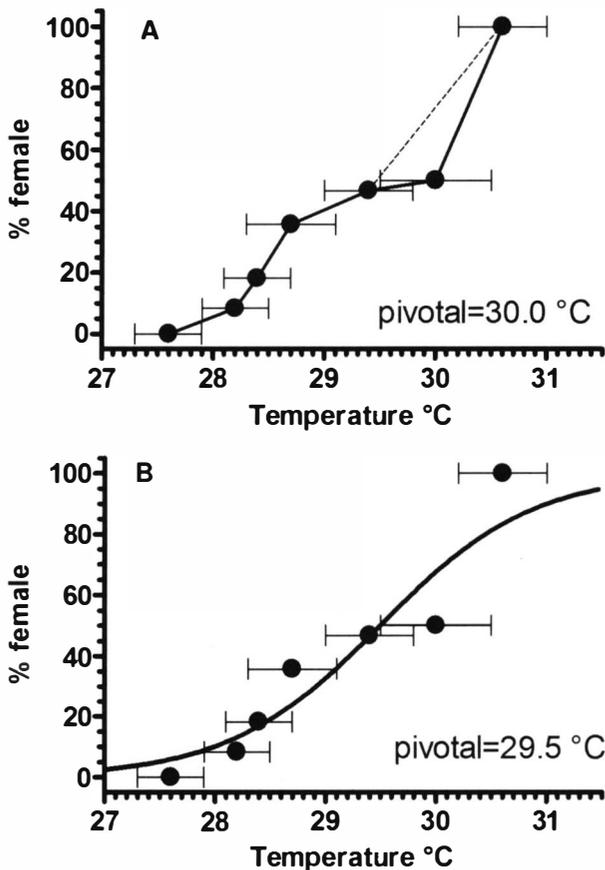


FIG. 1. Relationship between incubation temperature and sex ratio of green turtle eggs from Suriname. (A) shows how the data were plotted for the simple method of calculating pivotal temperature based on Mrosovsky & Pieau (1991). When the sex ratio data from the 30 °C group ($n=4$) were excluded, the simple method calculated a pivotal temperature of 29.5 °C (dotted line). (B) shows the sigmoidal curve fitted to the data by Prism4 software.

in the simple method of calculating pivotal temperature. Furthermore, the two other methods used to calculate pivotal temperature produced nearly identical values (29.4 and 29.5 °C). Recently developed standardized methods to analyze TSD data from reptiles (Girondot, 1999; Godfrey *et al.*, 2003) facilitate comparisons across TSD studies, although at the current time, we recommend that several methods be used to analyze the same dataset.

The pivotal temperature derived from this study (29.4 or 29.5 °C, depending on the method used) was slightly higher than the 28.8 °C value derived for the same population in an earlier study by Mrosovsky *et al.* (1984). There were some differences in the methods of the two studies; for instance, the earlier study employed a variety of different incubation substrates and also water was added more frequently to the eggs (McLean *et al.*, 1983). Overall, we were more confident of our methods employed in the current study, given its use of a standardized substrate and the wider range of constant incubation temperatures. Nevertheless, both studies were based on a small sample size (150 eggs from three clutches in 1983, 198 eggs from two clutches in 1995), and eggs of one clutch from the present study had a poor survival rate. Therefore, a more representative value of

the pivotal temperature of this green turtle nesting population might be based on a combined analysis of data from both studies. When we reanalyzed the data from both datasets (1983 and 1995), we calculated a pivotal temperature of 29.2 °C based on the simple interpolation method, 29.2±0.1SE °C based on the method of Girondot (1999), and 29.3±0.2SE °C, based on a sigmoidal curve fit with Prism4 (Fig. 3). Given the small discrepancy among the three methods to calculate pivotal temperature, 29.2 °C is the best estimate for this population at the current time.

Pivotal temperatures have been derived for some nesting populations of green turtles from around the world, using different methods of egg incubation (Table 2). Studying the sex ratio of hatchlings relative to nest temperatures on nesting beaches is valuable for gaining insight into the micro-environmental conditions specific to the study beach. However, studies involving field incubation of eggs for pivotal temperatures vary in methodology, particularly in terms of impact of substrate, hydric conditions, and nonrandom collection of a subset of hatchlings for classification of sex. This makes it difficult to compare pivotal temperatures across studies and populations. In contrast, laboratory incubation of eggs at constant temperatures is valuable as a more standard method that facilitates comparisons of results. Data from constant temperature experiments are also useful in developing “constant temperature equivalents” for interpreting variable temperature regimes recorded in natural conditions relative to hatchling sex (Georges *et al.*, 2004). Additionally, pivotal temperatures based on laboratory incubation and extrapolated sex ratios are basic life history characters that can be used in larger scale analyses, such as modeling of population dynamics (Crouse *et al.*, 1987; Chaloupka, 2002) and predicting population responses to environmental pressures such as global climate change (Mrosovsky *et al.*, 1984; Davenport, 1989).

Nevertheless, laboratory incubation of eggs remains far from being completely standardized among researchers and laboratories. For example, some researchers take into account evaporative cooling of eggs when presenting laboratory incubation temperature data (e.g. Mrosovsky, 1988; Godfrey *et al.*, 1999; this study) while others do not (e.g. Binckley *et al.*, 1998; Georges *et al.*, 1994; Wibbels *et al.*, 1998). Furthermore, some incubators used in the laboratory may have wide ranges around the mean “constant” temperature. For instance, a standard deviation of up to ±0.5 °C around the mean incubator temperature may translate into a temperature range of several °C (Limpus *et al.*, 1985). Cyclical temperature fluctuations about a mean temperature can, if large enough, result in more female hatchlings being produced than predicted by the mean non-fluctuating temperature (Georges *et al.*, 1994). A wide range of temperatures around the mean may explain the low pivotal temperature of <29 °C reported by (Miller & Limpus, 1981) in Table 2. Finally, there may also be differences across studies in the humidity levels

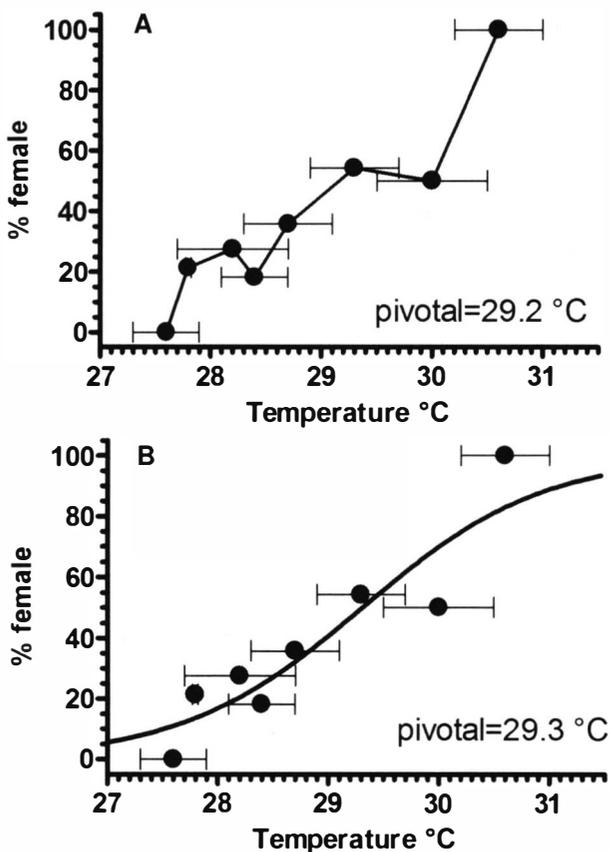


FIG. 3. Relationship between incubation temperature and sex ratio of green turtle eggs from Suriname, combining data from 1983 (Mrosovsky *et al.*, 1984) and 1995 (this study). (A) shows how the data were plotted for the simple method of calculating pivotal temperature based on Mrosovsky & Pieau (1991). (B) shows the sigmoidal curve fitted to the data by Prism4 software.

of the incubators or incubating substrate that can also affect sex during incubation (Steyermark, 1999), although the impact of thermal differences generally far outweighs those of nonthermal differences in incubation (Godfrey & Mrosovsky, 2001). Perhaps the best comparisons of pivotal temperatures across studies are restricted to data that are collected by the same laboratory using the same methodology. To date, in the laboratory used for the present study on green turtles, five other incubation studies have been conducted using nearly identical methods on clutches from: loggerhead turtles from USA, Brazil, and Greece (Mrosovsky, 1988; Marcovaldi *et al.*, 1997; Mrosovsky *et al.*, 2003), and hawksbill turtles from Antigua and Brazil (Mrosovsky *et al.*, 1992; Godfrey *et al.*, 1999). Interestingly, the pivotal temperatures from all these studies and species have been close to 29 °C, which conforms to the idea that pivotal temperatures in sea turtles are constrained around 29 °C (Mrosovsky, 1994).

However, there remain some qualifications of this claim of conservatism around pivotal temperatures in sea turtles. First, there are relatively few pivotal temperature data available to date, and the majority come from loggerhead sea turtles. It may be the case that in loggerheads, there is conservatism of pivotal temperatures around 29 °C, but the scant data available for species such as hawksbills, flatbacks, and green turtles make generalizations tenuous. Second, recent data from olive ridley sea turtles from Pacific Costa Rica reported that the pivotal temperature for this population is close to 31 °C (Wibbels *et al.*, 1998). Interestingly, a study on olive ridleys from India found a pivotal temperature of 29.5 °C (Mohanty-Hejmadi & Dimond, 1986). This could signal a wider variation of pivotal temperatures in olive ridleys or even other sea turtle species. However, it must be kept in mind that methodological variation in

the incubation methods may account for some or all of this higher pivotal temperature. For instance, there was no correction for evaporative cooling by (Wibbels *et al.*, 1998), although even adding a correction factor of -0.5 °C would still result in the highest pivotal temperature for sea turtles (Wibbels *et al.*, 1998). Also, the olive ridley eggs were incubated in groups rather than singly, which may have affected the metabolic warming produced during incubation. A better comparative test would be to incubate eggs of two or more different species and/or populations simultaneously in the same incubators using the same methodology.

Overall, there remains much work to be done on TSD in sea turtles, particularly in uncovering the nature of pivotal temperatures of different populations and species. We urge that more effort be spent on building up the database on pivotal temperatures. These data have a wider application, including forming a natural history character that can be used in population modeling and contributing to research about evolution (Rhen & Lang, 1998; Girondot & Pieau, 1999). They also have application for management and conservation, in terms of estimating the potential impacts on sea turtle sex ratios not only by local changes in nesting habitats (e.g. Mrosovsky *et al.*, 1995), but also by long term impacts such as increased feminization due to global climate change or conservation efforts (Janzen, 1994; Girondot *et al.*, 1998). Also important is the need to have data on TSD relevant to the local sea turtle population, rather than imported data from other regions or even ocean basins.

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TABLE 2. Values of green turtle pivotal temperatures from various populations, based on individual studies

Population	Pivotal temperature °C	Clutches	Method to estimate pivotal	Reference
LABORATORY STUDIES				
Suriname (1995)	30.0 (or 29.5)	2	simple method	This study
Suriname (1995)	29.5±0.2SE	2	sigmoidal curve fit (Prism4)	This study
Suriname (1995)	29.4±0.1SE	2	maximum likelihood curve fit	This study
Suriname (1983)	28.8	3	straight line	Mrosovsky <i>et al.</i> , 1984
Suriname (1983&1985)	29.2	5	simple method	This study
Suriname (1983&1985)	29.3±0.2SE	5	sigmoidal curve fit (Prism4)	This study
Suriname (1983&1985)	29.2±0.1SE	5	maximum likelihood curve fit	This study
Sarawak	<29.5	11	extrapolation	Leh <i>et al.</i> , 1985
Australia	<29.0	1	straight line	Miller & Limpus, 1981
FIELD STUDIES				
Costa Rica	28.0 to 30.3	33	unknown	Standora & Spotila, 1985
Ascension Is.	28.8	21	maximum likelihood curve fit	Godley <i>et al.</i> , 2002
N. Cyprus & Turkey	28.7	5	regression line	Kaska <i>et al.</i> , 1998
N. Cyprus	28.7, 29.2	48	regression line	Broderick <i>et al.</i> , 2000

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INFLUENCE OF SMALL-SCALE FIRES ON THE POPULATIONS OF THREE LIZARD SPECIES IN ROME

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The effect of small summer fires on three species of Mediterranean lizards (the lacertids *Podarcis muralis* and *Lacerta bilineata*, and the scincid *Chalcides chalcides*) were studied at five burnt transects and eight unburnt control transects in urban green areas of Rome. Overall, the fire had different effects on the three species at the local level of the single transects. *Lacerta bilineata* was not affected by fire treatment. *Podarcis muralis* showed a significant increase in numbers in two of five burnt transects. *Chalcides chalcides* declined after fire in all transects. The potential ecological causes for the observed patterns are discussed.

Key words: fire ecology, habitat disturbance, Rome, central Italy

INTRODUCTION

Fire may strongly influence the structure of habitats and of plant and animal communities (Thirgood, 1981; Whelan, 1995). In several ecosystems, including the Mediterranean evergreen chaparral (= macchia), fire is a natural regulator of the phytocenosis structure, as it occurs regularly during the dry summer (e.g. Marchand, 1990; Pantis & Mardiris, 1992). However, the effects of fire on a particular ecosystem will depend on their frequency, intensity, and season (Gill, 1975; Lemckert *et al.*, 2003). The effects of fire on vertebrates (e.g. reptiles) can be direct, i.e. by immediate killing of animals (Heinrich & Kaufman, 1985; Wilson, 1994; Whelan, 1995; Duck *et al.*, 1997; Homer *et al.*, 1998; Esque *et al.*, 2002), or indirect, i.e. by their modifications to the vegetation structure (Wilson, 1994; Brooks & Esque, 2002), with eventual ecological consequences for the animals, i.e. on their thermal attributes, microclimate (Hurlbert, 1969; Rice & Parenti, 1978), exposure to predators, food resource availability (Evans, 1984; Kaufman *et al.*, 1990), and timing of reproduction (Lillywhite & North, 1974; Withgott, 1996; Duck *et al.*, 1997; Cavitt, 2000). Although the effects of local fires on herpetofauna have received some attention (e.g. Lemckert *et al.*, 2003; and for a review see Russell *et al.*, 1999), unfortunately the studies on the effects of local fires on the population ecologies of Mediterranean reptiles are extremely scarce. Indeed, it is even hard to find any data on the fluctuations in the apparent numbers of reptiles in areas subjected to burning (but see Pinto *et al.*, 2006).

In this paper, we present data on the effects of small-scale fires on three sympatric lizard species (the lacertids *Podarcis muralis* and *Lacerta bilineata*, and the scincid *Chalcides chalcides*) along some line transects affected by fire, situated in urban green areas in the city of Rome (Central Italy).

MATERIALS AND METHODS

The data were collected by one of the authors (L. Rugiero) during surveys of the reptile communities of five nature reserves of Rome (Rugiero, 2004). Field surveys were carried out from March 2001 to May 2002. During the process of Rugiero's (2004) research, four out of five nature reserves were affected by small-scale fires. In two of these four areas ('Tenuta dei Massimi' and 'Insugherata' nature reserves; both with identical climate and vegetation characteristics), the fire only affected the transects which were already subjected to investigation. Hence, the data given in this paper were collected in the two above-mentioned nature reserves. The other two burnt nature reserves were not considered for this study because there were no transects which were directly affected by fire. All these fires, which affected an average area of about 3 to 5 ha surface in each study area, occurred during the summer months (August and September) of 2001.

Pooling the two nature reserves, five burnt line-transects (treatment in the following text: named A to E) were surveyed, both before and after fire, and eight unburnt transects at the same nature reserves were surveyed as controls (named (i) to (viii)). Both treatment and control transects were situated in the same nature reserves, i.e. they were under identical climatic characteristics. The minimum linear distance between transects ranged from 250 to 1600 m, and a habitat unsuitable for lizards was always present between two transects. Hence, all the transects were certainly independent of each other in terms of the lizard movements. All the treatment transects were affected by fire for their entire length, i.e. there was no portion of them which was not destroyed by fire. Three of the treatment transects (A, B, and D) were in the middle of the total burnt surface, whereas two transects (C and E) had one of the extremities which was near (< 15 m) the external limit of the burnt surface.

Each 300 m transect was surveyed once each research day (see Table 1 for exact dates of the field days at each transect) by walking very slowly in one direc-

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tion, and recording all the reptile sightings. Counts of all individuals of each species seen were recorded but lizards were not individually marked. Therefore individual lizards were counted only once per day but some individuals may have been counted on different days. Field surveys of the study transects were carried out during the same weeks in both treatment and control transects (Table 1), hence the eventual differences could not be caused by temporal oscillations of lizard population numbers. Moreover, all the transects were surveyed during sunny days, i.e. under nearly identical weather conditions.

A methodological limitation of this research study was that, using only visual observations of individuals, there may be some uncontrolled sources of bias for the results, as, for example, reptiles in burned areas may be more easily detected than those from unburned sites. This might artificially inflate the numbers of observed specimens. Unfortunately, this limitation is widespread in studies estimating population 'abundance' by visual sightings, not only in herpetology (e.g. Schmidt, 2004) but also in mammalogy and ornithology (Lebreton *et al.*,

1982; Nichols & Pollock, 1983; Link & Nichols, 1994). However, the use of control transects and the high visibility of the study species led us to suppose that this potential problem had not seriously compromised the validity of our conclusions.

TREATMENT TRANSECTS

Transect (A) was laid along the edge of a *Quercus suber* woodland, and was characterised by ecotonal scrub vegetation (*Spartium junceum*, *Prunus spinosa*, *Rubus ulmifolius*) and grassy vegetation. During the post-fire research period, there was no re-growth of the scrub vegetation, whereas the grass was slowly re-growing. Transect (B) was laid along a thick wood (*Quercus suber*). Underbrush (*Hedera helix*, *R. ulmifolius*, *Pteridium aquilinum*, and *Ruscus aculeatus*) was dense. The fire caused a strong reduction of the underbrush cover, but trees survived. Re-growth of the underbrush was mainly due to *P. aquilinum*. Transect (C) was laid along the edge of a bushland (*S. junceum*), with a continuous and well developed grassy cover. Fire occurred at the end of August 2001 and destroyed completely the

TABLE 1. Distribution of the dates for the field days of survey of each transect (both treatment and control), both before and after fire. Asterisks indicate dates of survey; F indicates dates of fire. For the transect symbols, see the text.

						Transect							
	A	B	C	D	E	i	ii	iii	iv	v	vi	vii	viii
Year 2001 (before fire)													
10 March				*	*								
22 March	*	*						*	*				
24 March			*	*	*					*	*	*	*
03 April	*	*				*	*	*	*				
09 April			*	*	*					*	*	*	*
18 April	*	*				*	*	*	*				
26 April			*	*	*					*	*	*	*
28 April	*	*				*	*	*	*				
04 May	*	*				*	*	*	*				
10 May			*	*	*					*	*	*	*
15 May	*	*				*	*	*	*				
28 May			*	*	*					*	*	*	*
Aug-Sep	F	F	F	F	F								
Year 2002 (after fire)													
20 March	*	*				*	*	*	*				
22 March			*	*	*					*	*	*	*
06 April	*	*				*	*	*	*				
07 April			*	*	*					*	*		*
21 April						*	*	*	*				
22 April	*	*											
25 April			*	*	*					*	*	*	*
01 May	*	*				*	*	*	*				
06 May			*	*	*					*	*	*	*
15 May	*	*				*	*	*	*				
21 May			*	*	*					*	*	*	*

TABLE 2. Means (\pm SD), and *t*-tests of the numbers of lizards observed along line-transects in Rome, before and after fire.

Species	Transect	Mean (\pm SD) Before fire	Mean (\pm SD) After fire	Change in abundance	<i>t</i> (df) mean	<i>P</i>
<i>Lacerta bilineata</i>						
TREATMENT TRANSECTS	A	6.33 \pm 2.2	4.40 \pm 1.9	1.93	1.50 (9)	0.167
	B	1.67 \pm 1.4	1.00 \pm 1.0	0.67	0.90 (9)	0.389
	C	3.0 \pm 3.24	0.6 \pm 0.9	2.40	1.60 (8)	0.149
	D	1.33 \pm 1.03	1.20 \pm 1.3	0.13	0.19 (9)	0.853
	E	3.67 \pm 1.7	2.20 \pm 1.6	1.47	1.42 (9)	0.189
CONTROL TRANSECTS	i	5.80 \pm 3.4	5.20 \pm 3.4	0.60	0.28 (9)	0.788
	ii	3.80 \pm 1.9	3.40 \pm 1.8	0.40	0.34 (8)	0.744
	iii	1.33 \pm 0.5	1.40 \pm 1.7	-0.07	-0.09 (9)	0.928
	iv	0.83 \pm 1.2	1.40 \pm 1.5	-0.57	-0.70 (9)	0.501
	v	2.80 \pm 1.8	5.40 \pm 4.9	-2.60	-1.12 (8)	0.296
	vi	3.60 \pm 2.9	1.39 \pm 0.9	2.21	1.63 (8)	0.141
	vii	5.00 \pm 2.9	5.00 \pm 2.3	0.00	0 (7)	1.000
	viii					
Overall comparison before vs. after fire		3.20 \pm 2.0	1.88 \pm 1.5	1.32	1.17 (8)	0.274
Overall comparison control transects		3.39 \pm 1.7	3.24 \pm 1.8	0.15	0.17 (14)	0.868
<i>Podarcis muralis</i>						
TREATMENT TRANSECTS	A	0.67 \pm 5.4	6.40 \pm 0.5	-5.73	-2.61 (9)	0.028
	B	3.00 \pm 2.1	9.20 \pm 3.9	-6.20	-3.38 (9)	0.008
	C	0.40 \pm 0.6	0.80 \pm 1.8	-0.4	-0.48 (8)	0.645
	D	3.67 \pm 3.0	6.80 \pm 3.3	-3.13	-1.63 (9)	0.136
	E	0.67 \pm 0.8	0.20 \pm 0.4	0.47	1.14 (9)	0.285
CONTROL TRANSECTS	i	1.60 \pm 2.1	3.60 \pm 2.1	-2.00	-1.52 (8)	0.166
	ii	0.40 \pm 0.5	0.80 \pm 0.8	-0.40	-0.89 (8)	0.397
	iii	3.00 \pm 1.8	2.80 \pm 2.3	0.20	0.16 (9)	0.874
	iv	8.67 \pm 4.7	9.00 \pm 6.6	-0.33	-0.10 (9)	0.924
	v	2.80 \pm 2.4	1.75 \pm 1.2	1.05	0.79 (7)	0.456
	vi	0.83 \pm 0.9	0.20 \pm 0.4	0.63	1.32 (9)	0.219
	vii	6.60 \pm 3.2	5.50 \pm 2.6	1.10	0.55 (7)	0.599
	viii	4.00 \pm 3.7	2.80 \pm 2.2	1.20	0.62 (8)	0.552
Overall comparison before vs. after fire		1.68 \pm 1.5	4.68 \pm 4.0	-3.00	-1.57 (8)	0.154
Overall comparison control transects		3.48 \pm 2.9	3.30 \pm 2.8	0.18	0.13 (14)	0.901
<i>Chalcides chalcides</i>						
TREATMENT TRANSECTS	A	3.17 \pm 2.0	0.40 \pm 0.9	2.77	2.8 (9)	0.021
	B					
	C	7.00 \pm 3.7	3.40 \pm 3.4	3.60	1.58 (8)	0.151
	D					
	E	2.33 \pm 2.6	0.20 \pm 0.4	2.13	1.81 (9)	0.103
CONTROL TRANSECTS	i	0.40 \pm 0.5	0.20 \pm 0.4	0.20	0.63 (8)	0.545
	ii					
	iii	3.40 \pm 3.2	2.80 \pm 1.1	0.60	0.39 (8)	0.703
	iv					
	v					
	vi	1.67 \pm 1.4	1.20 \pm 0.4	0.47	0.73 (9)	0.486
	vii					
	viii					
Overall comparison before vs. after fire		4.17 \pm 2.5	1.33 \pm 1.8	2.84	1.60 (4)	0.184
Overall comparison control transects		1.82 \pm 1.5	1.40 \pm 1.3	0.42	0.37 (4)	0.732

vegetation. During the post-fire research period, there was partial re-growth of the grassy cover. Transect (D) was laid along the border between a mixed wood (*Castanea sativa* and *Quercus cerris*, with underbrush formed by *P. aquilinum* and *R. ulmifolius*) and cultivated land. Fire occurred at the beginning of September 2001, and destroyed both the wood underbrush and the cultivation, whereas the trees survived. Re-growth of the underbrush, due mainly to *P. aquilinum*, started only after the third of the five post-fire research dates (see Table 1). Transect (E) crossed a Mediterranean bushland, with open grassy habitat dominated by *Asphodelus* spp. and *P. aquilinum*, with some *Cistus salvifolius* and rare *S. junceum* specimens. Fire completely destroyed the vegetation. During the post-fire research period, the re-growth of *Asphodelus* spp. was rapid, whereas that of *P. aquilinum* and of grassy sites were delayed; there was no re-growth of *C. salvifolius* and *S. junceum*.

CONTROL TRANSECTS

Transect (i) was laid along the border between a mixed woodland (dominant *Quercus cerris* and *Q. frainetto*, with also *Q. suber* and *Ulmus minor*) with ecotonal vegetation constituted by *R. ulmifolius*. Transect (ii) was laid along the border between a wide spiny bushland (*R. ulmifolius*) and a cultivated field. Transect (iii) was laid along the bank of a small stream, with some trees of *Populus nigra* and *Robinia pseudoacacia*, and bushy and grassy vegetation of *R. ulmifolius*, *Sambucus* spp. and *Urtica dioica*. Transect (iv) was laid along the wooded bank of a stream (trees were *Salix alba*, *R. pseudoacacia* and *P. nigra*), with a well developed cane-bed and with populations of *U. dioica*, *Conium maculatum* and *Silybum marianum*. Transect (v) was laid between the border of a mixed oak wood (*Q. pubescens*, *Q. suber*, *Q. ilex*, *Ulmus minor*) and a cultivated field; the ecotonal vegetation consisted mainly of *R. ulmifolius*, *U. dioica*, *Sambucus nigra*, *S. marianum* and *C. maculatum*. Transect (vi) was laid along a south-facing edge of a mixed oak wood (*Q. suber*, *Q. frainetto*, *U. minor*) with ecotonal scrub vegetation (*S. junceum*, *Prunus spinosa* and *Asphodelus microcarpus*). Transect (vii) was laid along the bank of a small and shady stream; the bank vegetation consisted of *Salix alba*, *S. nigra*, *U. dioica*, *R. ulmifolius*, *Equisetum telmateja*, *S. marianum*, and *C. maculatum*. Transect (viii) crossed a thick wood (*C. sativa*, *Q. robur*, *Carpinus betulus*, *Fraxinus ornus*), with relatively dense underbrush (*Ilex aquifolium*, *Ruscus aculeatus* and *Hedera helix*).

Statistical tests were done with a Statistica (version 6.4) PC package, with all test being two-tailed. The design of this study (choosing independent systems, i.e. the various transects; collecting data on each of them and then assigning them to a treatment and a control because of random events – fire) is normally adequate for a deductive experiment and minimises potential biases due to data pseudoreplication (despite the fact that liz-

ards were not individually marked; see Oksanen, 2001 for a discussion on this issue). Nonetheless, we also compared mean lizard abundance per day before the fire with mean abundance after the fire, using transects as replicates (and compared this with before- versus after-fire on unburnt controls). To be precise, for comparing burnt and control transects, we calculated the change in mean abundance before the fire to mean abundance after the fire for each transect; then we used 'change in abundance' as the dependent variable in the ANOVA model, and transects as replicates.

RESULTS

The data on the mean (\pm SD) numbers of lizards per day along the 300 m of transect, both before and after fire, for both treatment and controls, are given in Table 2. Post-fire means include counts on only burnt parts of the transect, because fire affected 100% of all the study transects (see methods). Although the numbers of lizards observed at each transect were often small, we were still able to identify some species-specific patterns.

The numbers of *Lacerta bilineata* tended to remain relatively stable after fire at all treatment transects, as well as in the control transects (means of the numbers of lizards observed per day were never statistically different before vs. after fire). Overall, the mean change in abundance of lizards was not significantly different before vs. after fire (ANOVA, $F_{1,10}=3.218$, $P=0.103$).

The numbers of *Podarcis muralis* were significantly higher after fire at transects (A) and (B), but not in the other three treatment transects and neither in any control transect. The mean change in abundance of lizards was significantly different before vs. after fire (ANOVA, $F_{1,11}=7.652$, $P=0.018$), with lizard numbers that increased after fire treatment.

The numbers of *Chalcides chalcides* were significantly lower after fire at transect (A), and were also lower (although not significantly) after fire in the other treatment transects, but not in the controls. The mean change in abundance of lizards was significantly different before vs. after fire (ANOVA, $F_{1,4}=29.792$, $P=0.0054$), with lizard numbers that decreased after fire treatment.

There was no effect of the year of study on the abundance of any of the three lizard species (all ANOVAs - for *L. bilineata*: $F_{1,23}=2.68$, $P=0.115$; for *P. muralis*: $F_{1,78}=0.01$, $P=0.912$; for *C. chalcides*: $F_{1,9}=2.04$, $P=0.187$).

DISCUSSION

Lacerta bilineata did not decrease noticeably after fire when compared to controls. We suppose that is related to two concurrent factors. First, this lacertid is extremely adaptable and occurs in a variety of habitats in Mediterranean Italy (Bruno & Maugeri, 1977; Corti & Lo Cascio, 2002; Rugiero, 2004). Second, it has a large home range (probably around 300 to 1200 m²; for the case of the sister-species *Lacerta viridis* see Saint Girons & Bradshaw, 1989), and therefore may escape

fire and recolonise the burnt habitat efficiently and in a short time-span.

Overall, *P. muralis* was in part positively affected by fire (in comparisons of treatment vs. control transects). Indeed, there was a significant rise in numbers after fire in transects (A) and (B), and also the mean change in abundance of lizards showed that the numbers of these lizards tended to increase significantly after fire. These positive effects on lizard numbers by fire were likely local trends related to the effects of fire on the site characteristics of the study transects. Indeed, these rises in lizard numbers were probably caused by the sudden opening of new clearings for thermoregulation in the closed wood, which is the typical habitat for *P. muralis* in the study area (Rugiero, 2004). Obviously, these data do not imply that *P. muralis* may benefit from large-scale fires. Our data just indicate that this species may resist small-scale fires better than the other two study species, and that it may derive some advantage from small scale fires, especially areas of closed vegetation.

Chalcides chalcides was negatively affected by fires in all treatment transects (however, the difference before and after-fire was statistically significant in just one transect), and the mean change in abundance of lizards showed an overall statistically significant decreasing trend after fire. As there was no evidence of decline after-fire in any control transect, we are led to believe that fire may really be detrimental to *C. chalcides*, at least when it entirely removes large portions of the grassy vegetation layer which is this species' typical habitat (Rugiero, 1997).

In conclusion, this study confirms previous research on other animals (e. g. Orgeas & Ponel, 2001) indicating that the species which are more versatile in terms of habitat requirements survived the devastations produced by fires better (*L. bilineata* and especially *P. muralis* in our study areas). However, it must be stressed that the situation may be different with large-scale fires.

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REVIEW OF THE REINTRODUCTION PROGRAMME OF THE MUGGER CROCODILE *CROCODYLUS PALUSTRIS* IN NEYYAR RESERVOIR, INDIA

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Human-crocodile conflicts created by Mugger crocodiles *Crocodylus palustris* were studied 18 years after a reintroduction to the Neyyar Wildlife Sanctuary, Kerala, India. Twenty-nine Mugger crocodiles were reintroduced into the reservoir in the year 1983 and crocodile attacks on livestock were reported from 1985. During the initial period of the study, 21 to 25 Mugger crocodiles were estimated but only 10 to 16 crocodiles were recorded towards the end of the period as nine were removed from the reservoir to reduce the conflict. Fishes provided sufficient prey, but food in the form of large mammals was inadequate. Twenty-nine crocodile attacks on humans were reported prior to the study and six occurred later, including two fatalities. The attacks occurred over 26 km of shoreline and followed previous patterns of attack behaviour in crocodiles. Larger crocodiles were more often involved with attacks than small crocodiles. About 2808 houses exist in a narrow belt near the lake shore. As local people utilised the reservoir for various purposes they did not support the conservation of crocodiles in the present circumstances. The case study indicated the failure of the reintroduction programme of Mugger crocodile in the Neyyar Reservoir.

Key words: crocodylian, human-wildlife conflict, prey availability, reintroduction, reptile

INTRODUCTION

Crocodylians have benefited from protection and strict control measures. As a result, crocodylian populations have increased and ranges have expanded back into historically occupied areas. This has also brought about an increase in the number of large crocodiles, and thus increasing conflicts between crocodiles and people and their livestock. In the Indian subcontinent, three species of crocodiles occur, the gharial (*Gavialis gangeticus*), the saltwater crocodile (*Crocodylus porosus*) and Mugger crocodile (*Crocodylus palustris*). The Mugger crocodile is distributed in most parts of India except Jammu and Kashmir and some northern Indian States (Whitaker & Daniel, 1978).

Thirty-six Mugger crocodiles were reintroduced into the reservoir of the Neyyar Wildlife Sanctuary in 1983 as a part of the crocodile conservation project launched in the State of Kerala with the joint effort of the Government of India, U.N.D.P. and F.A.O. of the United Nations. Crocodiles were present but rare in the Neyyar river system before the reintroduction programme, but the future of the population was bleak due to the animosity of the local population. From 1985 onwards, the crocodiles started attacking local inhabitants along the bank of the reservoir and many people were injured in the process. Further reintroductions of Mugger crocodile in the reservoir were stopped. As human-crocodile conflicts increased, a study was initiated in the year 2000 to evaluate the reintroduction program.

Early literature on crocodiles in India mainly dealt with the biology of the species and documentation of folk-

lore (D'Abreu, 1915; McCann, 1935; Dharam, 1947). De Vos (1982) prepared a manual on crocodile conservation and management in India, which formed the basis for crocodile conservation in India. Ross *et al.* (2000) discussed the problems of success in crocodile conservation. After the reintroduction program of crocodiles into the wild, many reports have appeared based on the programme from India. Acharjyo (1978) reported on the return of Mugger crocodile to the wild. Similarly, many authors reported on aspects such as conservation (Bustard, 1975; Chaudhury & Bustard, 1975), sexing of crocodiles in captivity (Kar & Bustard, 1979), growth of captive crocodiles (Krishnamurthy & Bhaskaran, 1979; Krishnamurthy, 1980; Bustard & Chaudhury, 1980; 1981), attacks on domestic livestock and man (Kar & Bustard, 1981; 1983), food requirement and movement (Singh, 1984a,b; Rao & Chaudhury, 1992) and other issues (Sagar & Singh, 1993; Kumar *et al.* 1999; Pillai, 1999). However, no detailed study was carried on human-crocodile conflicts created by the reintroduced crocodiles or evaluated any of the reintroduction programmes.

The objectives of this study were to evaluate the reintroduction programme by assessing the present population status of crocodiles in the Neyyar Reservoir, to study and characterize the circumstances under which crocodiles attacked humans and to assess the response of the local community towards crocodile conservation.

STUDY AREA

The Neyyar Wildlife Sanctuary, declared in 1958, is situated in the Thiruvananthapuram District in Kerala State, India (Fig. 1). The extent of the sanctuary is 128 km² and lies between 8° 17' and 8° 53' N latitudes and

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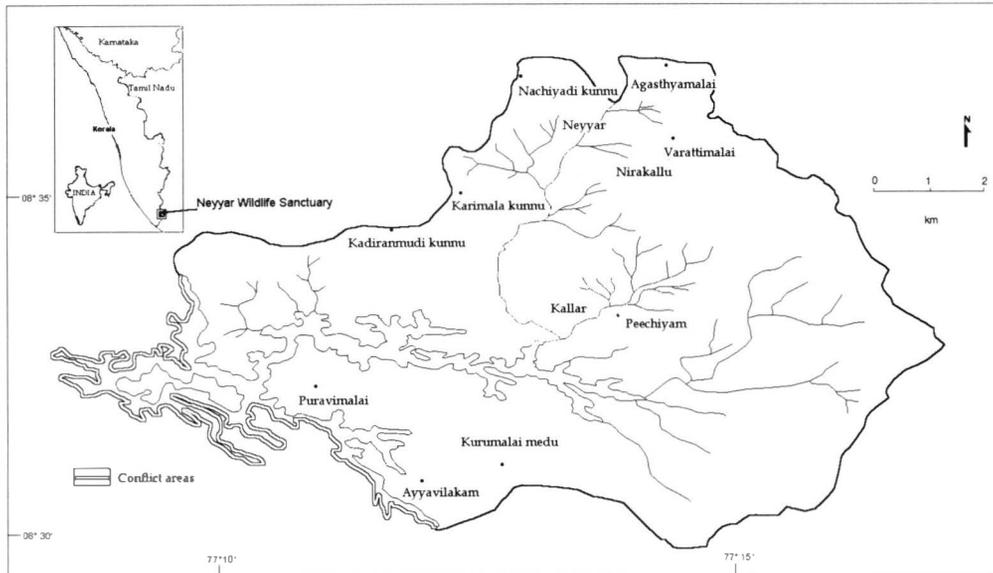


FIG 1. Neyyar Wildlife Sanctuary showing human-crocodile conflict areas

between $76^{\circ} 40'$ and $77^{\circ} 17'$ E longitudes, and is situated at the southern tip of the Western Ghats. A dam was built in the Neyyar River in the early 1940s for the purpose of irrigation and the area of the reservoir is 8.45 km^2 . The entire sanctuary area is rugged with undulating terrain and many rivers, streams and their tributaries and the total shoreline length is 107 km.

The major vegetation types in the sanctuary are west coast tropical evergreen, southern hilltop tropical evergreen, west coast semi-evergreen and southern moist mixed deciduous forests (Champion & Seth, 1968). The climate is tropical with heavy rainfall and high temperature and both south-west and north-east monsoons are prevalent, although maximum precipitation is derived from the north-east monsoon during the month of September. Temperatures vary from 16°C to 35°C and water level in the reservoir varies during different months. Except for the months of March to June, the water level was above 80 m and lowest level of water was found during the month of May.

MATERIALS AND METHODS

POPULATION ASSESSMENT

Population counts were made in the whole reservoir by direct sightings and by indirect evidence by walking along the banks of the reservoir and from boats including all the rivers and creeks. Crocodiles above 1.6 m in size were categorised as adults and those between 1.2 m and 1.6 m as subadults (Arumugam & Andrews, 1993). In each month, all the areas of the reservoir were visited and direct and indirect sighting of crocodiles were recorded. Indirect evidence was the presence of footprints, and body and tail imprints on the bank of the reservoir. A total count census (night and daytime count) was done in the month of March 2001 with the help of the staff of Kerala Forest Department and volunteers. Night counts were made using searchlights from a rowing boat and the light reflecting from the eyes of the crocodile helped to detect the animal.

ASSESSMENT OF PREY AVAILABILITY

Fishes. Fish samples were collected using gill nets of varying mesh sizes ($2 \times 2 \text{ cm}$ and $5 \times 5 \text{ cm}$), from representative locations on different days, closer to the area, where human-crocodile conflicts were reported. Since there is no regular fishing activity in the reservoir, random catches were made in order to get an estimate of possible catch during a year. The large mesh sized net was 100 m long, whereas the short mesh sized net measured 150 m. Gill nets were spread across the reservoir at 1800 hr and fishes were collected at 0600 hr. Cast nets were not employed because this method was not prevalent in the area. The number of *Catla catla* and *Labio rohita* fingerlings released into the reservoir was collected from the Deputy Director, Department of Fisheries, Thiruvananthapuram. The amount of fish caught in a day by the unauthorised fishing was also estimated by assessing the quantity of fish transported in a day by illegal catchers from the reservoir.

Mammals. Availability of wild mammals in the forest was assessed using indirect methods by identifying scats, pellets and spoor. Fifteen transects of 100 m length were searched for indirect evidence of smaller mammals in each month. Transects were placed at different localities in the sanctuary distributed all around the reservoir. During the fieldwork whenever an animal was sighted, it was identified and details of species and number were recorded. Diet of the crocodiles was studied by examining the scats collected from the reservoir banks. Even though the sample was small, some inferences can be made. Scats were soaked in water for 48 hr and separated using sieves and then the animal parts identified.

HUMAN CROCODILE CONFLICTS

Information on human-crocodile conflicts in the Neyyar Wildlife Sanctuary was quantified by interviewing the victims and by visiting the site where the attack occurred. All the applications filed in the Office of the

Assistant Wildlife Warden, at Neyyar were scrutinised for details and the fifteen human-crocodile encounters that were serious and occurred before the study were assessed in detail after visiting the victims. In addition, a detailed structured questionnaire survey was also carried out on the socio-economic aspects of the local people within 800 m from the reservoir. The questionnaire consisted of 34 questions with sub divisions *viz.* identification details, profile of respondent, dependence of reservoir, conflict with crocodile, source of drinking water, details of attack, house and infrastructure and 150 families were sampled for the survey.

POPULATION PRESSURE ON THE RESERVOIR

The dependence of local people on the reservoir for drinking water, bathing, washing clothes etc., was assessed from 0600 hr to 2000 hr in each month by direct observation. The number of people engaged in different activities in the reservoir was counted by walking through the banks of the reservoir during different times. An assessment of the number of houses near the reservoir in the selected sample portions was also carried out. Ten stretches of 1 km each were selected systematically along the bank and the number of houses in each stretch was assessed within a distance of 400 m from the reservoir to the families were also ascertained from the survey.

RESULTS

STATUS OF THE CROCODILE POPULATION

Based on sightings, the number of Mugger crocodiles in the sanctuary was estimated as 25 to 35 animals during January 2001. In the total count census, 12 Mugger crocodiles were found adjoining human habitations and two animals in the interior areas. An adult with 10 juveniles was recorded in the month of May 2000. Most of the crocodiles sighted were adults of more than 3 m in length and only a few subadults were recorded. Crocodile eggs were found in the sanctuary during the breeding season of 2000 and 2001. However, during 2002, no eggs were recorded. Even though hatchling and juveniles were recorded in May 2000, no hatchlings or juveniles were recorded in 2001 and 2002.

TABLE 1. Number of crocodiles recorded in the Neyyar Reservoir during May 2000 to December 2001.

Months	No. of crocodiles	Size class
May 2000	5	Adult
	8	Hatchlings
October 2000	2	Adult
November 2000	2	Adult
December 2000	3	Adult
January 2001	2	Adult
February 2001	1	Adult
March 2001	14	Adult
May 2001	4	Adult
June 2001	4	Adult
July 2001	1	Adult
August 2001	5	Adult
	1	Subadult
September 2001	1	Subadult
	3	Adult
October 2001	2	Subadult
December 2001	2	Subadult

The results indicated that sightings of Mugger crocodile were low and there was no significant difference in sightings of them between wet season (June-December) and dry season (January-May; $t=0.80$, $P=0.45$, $df=8$). Apart from the adults, only eight hatchlings and one subadult were recorded. Detailed sighting records of Mugger crocodile in each month are given in Table 1. Nine faecal samples of Mugger crocodiles were collected from the bank, which provided indirect evidence of crocodiles in certain areas and helped in the estimation of the population. After the two casualties in January 2001 and August 2001, nine large Mugger crocodiles were caught from the reservoir and four died for various reasons. All these Muggers except one were more than 3 m in length. Taking this into consideration, it was estimated that only 10 to 16 Mugger crocodiles were left in the wild at the end of the study period. Some crocodiles might have been poached when the two human casualties occurred.

TABLE 2. Summary of fish sampling from the Neyyar Reservoir.

Sl. No.	Station	No. of days sampled	Total fish caught (g)	Daily average (g)	Estimated annual catch (kg)
1	Safari park	3	4120	1373	501
2	Aruvipuram	3	8500	2833	1034
3	Mullayar	3	12,410	4137	1510
4	Kombai	4	16,000	4000	1460
5	Kottampuram	4	5650	1413	516
6	Boat landing	3	4750	1583	578
7	Puravimalai	2	950	475	173
8	Karumankulam	1	0	0	0
Total		23	52,380	2277	5772

PREY AVAILABILITY

Abundance of fish. Twelve species of fishes were collected from the reservoir. Most of the larger fishes collected were in the range of 1-2 kg. These were *Labio rohita*, *Catla catla*, *Cirrhina mrigala*, *Channa striatus*, *Wallago attu*, *Oreochromis mossambicus*, *Etroplus suratensis*, *Barbus* sp., *Puntius thomassi*, *Puntius filamentus*, *Puntius sarana* and *Cyprinus carpio*.

Neyyar Reservoir is a protected area and no regular fishing is allowed. Hence, there is no information available on the fishery potential of this reservoir. However, the Department of Fisheries, Kerala has been stocking fish hatchlings here, mainly large carp under the Indo-German project. The most recent stocking was done in 1995-1996 and 1996-1997. According to data supplied by the Department, during 1995-1996 16,38,050 fingerlings and in 1996-1997 5,39,542 fingerlings were introduced respectively. Crocodiles are known to prefer scaleless variety of fishes. *Wallago attu* is a scaleless variety which was found only rarely in the samples. Species of fish that are abundant in the reservoir are *Catla catla* and *Oreochromis mossambicus* both of which possess scales.

An estimated catch of 5772 kg of fish can be obtained from the reservoir (Table 2). In addition to this, we determined by enquiry that almost everyday local and tribal people catch fish from the reservoir for their own consumption or for local sale. Details were not available as these are unauthorised catches. However, from the information obtained through oral enquiry, these people catch anything between 1 to 12 kg per day. Based on the data gathered from the locals, an estimated average catch of 4 kg is caught per day, making a total of 1460 kg per year. This information was gathered on the same days on which the sample surveys were made. Hence, it may be added to the total catch and a grand total arrived at as 7232 kg of fish per year. Evidently, the potential catch may be several times this as the estimate is based on a small sample size.

Abundance of mammals. An attempt was made to assess the abundance of mammals on the banks of the reservoir. Sambar deer (*Cervus unicolor*) and bonnet macaque (*Macaca radiata*) were recorded on many occasions. An incident of a Mugger crocodile attacking a sambar and another incident of a Mugger crocodile attempting to catch a bonnet macaque were reported. Indirect evidence on the presence of sambar, wild boar

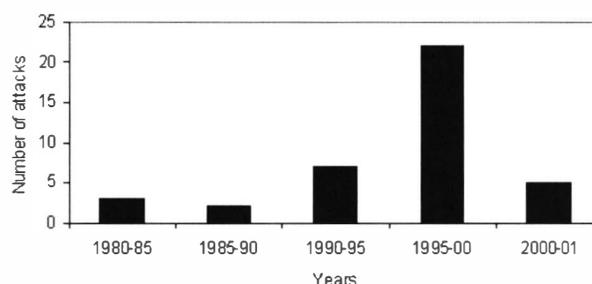


FIG. 2. Number of crocodile attacks in different years.

(*Sus scrofa*) and bonnet macaque were also obtained from the line transects.

Line transect direct counting was not attempted as sightings of large herbivores were very few. Crocodile scat analysis revealed the presence of sambar hairs in two samples. No other food materials could be identified from the scats. Other than wild animals, crocodiles were feeding on livestock and domestic animals, namely stray dogs, small cows, goats, buffalo calves, domestic fowl, ducks and cats. Apart from fishes and mammals, crocodiles are known to feed on birds. Only few species of aquatic birds were recorded from the reservoir and migratory ducks were absent. The little cormorant (*Phalacrocorax niger*) was the most abundant bird species in the reservoir and ten to twenty birds were seen in a flock. No predation on little cormorants by crocodiles was recorded in this study. Apart from little cormorant, darter (*Anhinga rufa*) and little egret (*Egretta garzetta*) were also seen rarely in the reservoir.

HUMAN-CROCODILE CONFLICTS

Past attacks on humans. Crocodile attacks were reported from 1985 onwards, and before the beginning of the study, 30 incidents were recorded. Among these, more than 15 were serious attacks on humans, involving ten men and five women (Fig. 2). A woman was attacked twice causing severe injury to body and hand and in another incident, a woman lost her forearm. Similarly, many victims survived crocodile attacks and live with serious deformities. All other victims were severely injured and hospitalised for periods varying from one to six months. Most of the attacks were on the legs and the attacks happened when the victims were in knee-deep water for bathing or for washing clothes. Two peaks of attacks were noticed, one in the morning hours and the other in the evening. The age of the victims ranged from

TABLE 3. Crocodile attacks on humans during the study.

Date of attack	Name of person	Age	Time of attack	Compensation received (Rs.)	Activity of the victim
10.10.2000	Ms. Rosamma	60	08.00 am	Hospital expenses	Washing (Lost hand)
2.1.2001	Ms. Rajamma	57	06.30 am	50,000	Washing (Killed)
7.5.2001	Ms. Chinnamma	40	09.00 am	Nil	Bathing (Lost basket)
16.5.2001	Mr. Surendran	42	08.30 am	Hospital expenses	Bathing
16.8.2001	Mr. James	56	06.00 am	50,000	Washing face (Killed)
29.9.2001	Mr. Ajesh	20	08.30 pm	Nil	Bathing

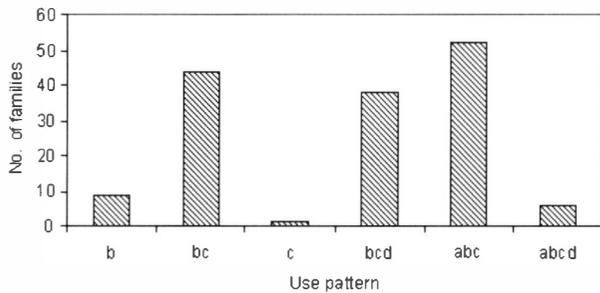


FIG. 3. Dependence of local people on Neyyar Reservoir: a, drinking water; b, bathing and washing clothes; c, washing of household materials; d, bathing of cattle.

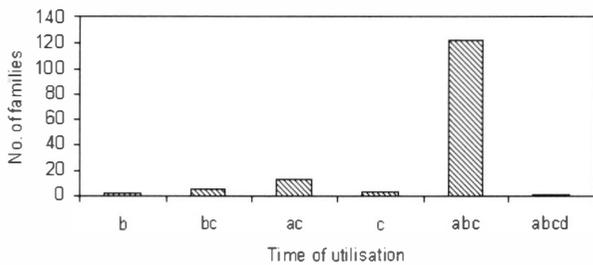


FIG. 4. Daily utilisation pattern of the Neyyar Reservoir: a, 0500 to 1000 hr; b, 1000 to 1300 hr; c, 1300 to 1600 hr; d, 1600 to 1830 hr.

8 to 60 years. According to local people, many attacks on livestock occurred after the release of the crocodiles into the reservoir, some of which were not reported to the officials. Many of the victims were alone when the attack happened. The chronology of attacks revealed that highest number of attacks occurred during the years 1995 to 2000.

Attacks on humans during the study. During the period of study, six crocodile attacks happened and two people died, whereas in the earlier incidents, no human casualties were reported (Table 3). The woman killed in the second attack and the man attacked in the fourth incident were following a regular pattern of activity, i.e. going for washing the clothes or bathing every day at a

particular time in same place. In the fifth attack, the man was unaware of the crocodiles in the reservoir and went to the reservoir alone in the morning; the body of the victim surfaced only on the next day. No specific time was observed in the pattern of attacks. The incidents of attacks were recorded from Kappukad to Kumbichal within a stretch of 26 km, but most people were injured near the dam site and Pantha within this stretch. In some cases, there is a relationship with the dumping of waste food in the lake. Following a regular pattern of activity might have helped the crocodiles to locate the humans for attack and wait for their arrival. All the attacks followed the known pattern of hunting behaviour reported in crocodiles (Daniel, 1983). As seen from the case studies, large crocodiles above 3 m length were involved in all the major and fatal attacks on humans.

Population pressure. A major factor contributing to the human-crocodile conflict is the proximity of human habitations to the reservoir. Local people were utilising the reservoir in a stretch of 26 km starting from Kappukad to Kumbichal (Fig. 3). Presence of people in the vicinity of the reservoir occurred between 0500 hr and 2300 hr. However, the majority of families (83%) were using the reservoir from 0500 hr to 1600 hr. During the study period, about 35 people were observed utilising a 3 km stretch of the reservoir in the morning (0600 hr to 0700 hr).

People utilise the reservoir for various daily needs, including washing, bathing, collecting water, washing cattle and retting of coconut leaves (Fig. 4). Apart from these, people cross the reservoir for collecting grass to be used as fodder and for gathering firewood. As many of the private holdings are surrounded by the waters of the reservoir, people cross the reservoir using traditional boats and two ferry services were operating across the reservoir. The number of households in the sampled areas is given in Table 4, which shows a high density of houses near the reservoir. Indeed, the main source of drinking water is the reservoir. The mean number of

TABLE 4. Number of households near the vicinity of Neyyar Reservoir.

Sl. No.	Locality stretch of 1 km	Distance from the Reservoir (m)				Total number of houses
		100	200	300	400	
1	Kappukad	0	0	6	6	12
2	Mlavatti	0	0	2	13	15
3	Neyyar Dam	109	12	7	2	130
4	Marakunnam	132	30	0	12	174
5	South Pantha	73	23	6	6	108
6	Mayam	77	20	12	30	139
7	Parathi	48	22	2	4	76
8	Kumbichal	70	45	40	14	169
9	Near Dam	35	40	52	72	199
10	Puravi Malai	24	2	8	23	57
Total		568	194	135	182	1079

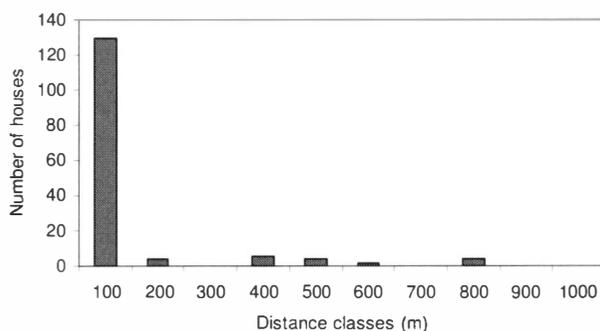


FIG. 5. Distance of houses from the Neyyar Reservoir.

houses in a one kilometre stretch was 108 and the maximum number of houses was seen near the dam site followed by Kumbichal, and Marakunnam. If we extrapolate this into 26 km of inhabited shoreline, 2808 houses are estimated to exist within 400 m of the reservoir.

Socio-economic status. The 150 families surveyed comprised 358 men and 334 women. Only 35 families were ready to move out in order to escape from the attacks of crocodiles. Most families were educated below high school level (453); others included members educated to high school level (160), higher secondary (57), and degree (23). Houses owned by low income families had reed roofs (30), while those of middle class families possessed asbestos roofing (53) and tiles (56). Other types of roof were only 12 in number. In terms of income, the families depended on daily wage labour for their livelihood (133), followed by farming (13). Of the surveyed houses, 130 were located within a 100 m of the reservoir (Fig. 5).

LOCAL ATTITUDES TOWARDS THE CONSERVATION OF CROCODILES

Sixty-one percent of the local people believed that the relocation of crocodiles from Neyyar Reservoir was the most suitable solution to the problem. Thirty-three families have no opinion and 17 families (11%) favoured killing the crocodiles. Nobody supported the idea of conserving the crocodiles in the Neyyar Reservoir in the present circumstances. Other methods, such as constructing fences were suggested by seven families; supplying drinking water through pipes was preferred by only one family, and relocation of families was suggested by one family. Sixty-one families (41%) depend on the reservoir, while 87 families depend on wells; two families depend on streams and one family on a pond. All the families raise livestock and 28 families have cattle, 38 have cats, 75 have dogs, 17 families have goats and 75 families have chickens.

DISCUSSION

The crocodiles were easily spotted when they basked on the banks of the reservoir and when they were swimming in the lake during the daytime. The best months for censusing the Mugger crocodile in Neyyar Reservoir are April and May, when the water level is the lowest and

banks are exposed to the maximum. By selecting these months, the basking crocodiles can be detected easily. In wild populations of crocodiles, natural mortality was very high and many juvenile crocodiles were entangled in the fishing nets and drowned in the reservoir. Additionally, local people destroyed crocodile eggs whenever they located them in the sanctuary. As a result, recruitment to the population of crocodiles is low or almost zero at Neyyar.

The density of herbivores was low in the Neyyar Wildlife Sanctuary, which could be attributed to a number of possible reasons. The history of the area shows that 111 ha of the sanctuary were planted with *Eucalyptus* hybrids in 1964 and 1965 (Vighnarajan, 1990). The planting operations and subsequent felling have reduced the density of herbivores and the remnants of the *Eucalyptus* plantations are seen in many places. Apart from this, the Kani tribals living in the sanctuary are hunter-gatherers and they hunt many small animals. In a previous study, this was confirmed from the adjacent Peppara Wildlife Sanctuary (Jayson, 1998). An adult wild crocodile with 3.5 m length, weighing 200 kg, may need 100 to 200 kg of food per year comprising all animals based on the assumption that the animal may consume 1-2% of body weight per week (Ross, 2000). From the analysis, it can be concluded that food in the form of fishes is sufficient, whereas food in the form of large mammals may be inadequate.

Possible reasons for attacks. One of the possible reasons for attack on people was territorial defence. From October to December, the males will be courting the females and intruders into the territory – including humans – are usually attacked. Again, in the months from February to May, the females will be laying eggs and defending nests, and attacks are more common. From June to September the females will be protecting the nestlings and chances of attacks are more by females during this time. Presence of livestock and other domestic animals on the banks may have attracted crocodiles to inhabited areas. In addition, the dumping of waste food materials on the banks of the reservoir provides an added attraction for the crocodiles. As the crocodiles grew, the number of attacks also increased, showing that the size of the crocodiles also contributed to the human-crocodile conflict.

The high human population density on the banks of the reservoir contributes to the human-crocodile conflict. It is not practical to relocate families from the banks as – with the exception of a few houses on the revenue land – all the owners had genuine land records. The analysis revealed that local populations heavily depended on the reservoir for their daily needs and most of the families depended on daily wage labour for their livelihoods. In the opinion of local people, removing the crocodiles from the reservoir by catching them or by killing them is the only way to solve the problem.

According to Richard Ferguson, Vice-chairman (Africa), IUCN Crocodile Specialist Group, the Zimbabwe programme with *C. niloticus* was the only major reintro-

duction programme carried out in Africa. It ran for five years between 1991 and 1995, but was assessed as largely unnecessary and could have been implemented more successfully on a smaller scale in better selected areas. In Africa also there have been a small number of incidents in which released animals have subsequently been shot for interfering with livestock and human use of the water bodies. The majority of the animals released in Zimbabwe during this programme will not yet have reached a size that is a real threat to humans.

CONCLUSIONS

Human attacks by crocodiles cannot be related to scarcity of food. Crocodiles of a sufficiently large size may attempt to prey on larger animals including humans in vulnerable locations independent of other food sources. Extremely well fed crocodiles with an abundance of natural prey might be less likely to prey on people, but this is uncertain. The relative size of prey, prey behaviour and particularly the apparent vulnerability of prey, hunger level, temperature, season, time of day and densities may be involved in a crocodile's 'decision' to carry out an attack. People, particularly those of smaller stature (women and children) at the water's edge or in the water, distracted by other activities (washing, fishing), following predictable daily patterns of movement and becoming complacent about crocodiles are likely to be attacked sooner or later.

The best solution is to change people's behaviour so that they are unlikely to encounter crocodiles in the crocodile's habitat. The provision of enclosures within which people can access the water's edge in safety to wash, collect water etc. is not feasible at Neyyar due to the long distance and varying water table and the steepness of banks. It is possible to manipulate the size distribution of the crocodiles by removing some of the larger and more dangerous individuals to other reservoirs in the State (Ross, 1998). Walsh & Whitehead (1993) also suggested capturing problematic crocodiles for relocation as a management strategy in Australia. Another strategy to manage the crocodile populations is to treat them as a sustainable resource (Brazaitis, 1983), which is not possible here unless the wildlife protection rules are changed. Due to social commitments, it is not practical to relocate people from the fringes of the Neyyar Reservoir to other areas. Except for a few recent settlements on the bank of the reservoir, the majority of the people have been living there from before the crocodiles were released into the reservoir. From our studies, it is clear that even if the local population is provided with drinking water they will continue to utilise the reservoir for bathing, fishing and washing of cattle. During summer, people from distant places also depend on the reservoir for drinking water and bathing.

As the local inhabitants dispose of waste food materials, including meat, into the reservoir, there is an added attraction for crocodiles to be near human habitations rather than the interior forests. As the herbivore population is low in density, crocodiles are always attracted

towards the populated areas from where easy prey like dogs, cows, goats, poultry and discarded waste food are easily available. This case study indicated the failure of the reintroduction programme of Mugger crocodile in the Neyyar Reservoir.

Based on this study, the following management strategies have been suggested to mitigate the problems connected with human-crocodile conflict in the Neyyar Wildlife Sanctuary. Assessment of the population of crocodiles in the reservoir is a prerequisite for any management decision. It is recommended to monitor the crocodile population in the Neyyar Reservoir to identify and selectively remove problem animals. Any crocodiles more than 3 m in length seen near the inhabited areas are potential attackers on humans. It is recommended to capture the crocodiles longer than 3 m from the Neyyar Reservoir and maintain them in captivity. Effective public awareness programmes with training, surveys, education and taking steps to reduce the chances of attacks should be initiated to save the crocodile population in the reservoir.

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PRESENCE AND ABSENCE OF THE CEMENT GLAND IN FOAM-NESTING LEPTODACTYLIDS (ANURA: LEPTODACTYLIDAE): IMPLICATIONS FOR THE TRANSITION TO TERRESTRIAL DEVELOPMENT

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Reproduction and early development are compared in three foam-nesting leptodactylids: *Leptodactylus fuscus*, *L. validus* and *Physalaemus pustulosus*. *Physalaemus pustulosus* and *L. validus* produce floating foam nests containing large numbers of small eggs which hatch early and soon leave the nest as larvae, attaching to solid surfaces by their cement glands until the stage of independent feeding. *Leptodactylus fuscus* foam nests are deposited in terrestrial burrows and contain small numbers of large eggs which hatch early, but remain in the foam nest until the yolk is resorbed and the larva has developed its tail for effective locomotion. The lack of a cement gland in *L. fuscus* suggests that the post-hatching period in the nest is a normal part of development. *L. fuscus* eggs and hatchlings transferred prematurely to water displayed low survival. The results are discussed in the context of evolutionary reproductive transitions within the leptodactylids.

Key words: cement gland, foam-nest, frog reproductive strategy, Trinidad

INTRODUCTION

The cement gland is a transient embryonic and early larval structure in anuran amphibians, first seen before hatching at around Gosner (1960) stage 14-15 and usually disappearing around the onset of feeding, at stage 25-26. Its role is to produce a sticky secretion that allows newly hatched larvae to hang motionless from egg capsules, water surface films or structures in water such as plant stems and rocks: during this period, external gills regress and mouthparts develop until the larva is ready to feed (Sive & Bradley, 1996).

However, not all anurans have a cement gland. Duellman & Trueb (1986) noted that embryos with prolonged intra-capsular development and a late hatching stage may have transient cement glands, and that direct developing species, such as the eleutherodactylids, may lack them altogether. Thibaudeau & Altig (1999) expected that cement gland loss should be a general feature in endotrophic anurans – i.e. those that develop the adult form without feeding on external sources – and reported that studies so far have shown an absence of cement glands in endotrophic species not developing in a nest (such as viviparous and marsupium-brooded species) and that cement glands are often not functional in nest-laying endotrophs.

Cement gland presence or absence and morphology are rather rarely reported in general accounts of anuran characteristics, probably because of the gland's transience, but it is clearly a key feature in evolutionary transitions from aquatic to non-aquatic modes of reproduction. In a study of comparative development and

morphology of the cement gland in 20 anuran species from six families, Nokhbatolfighahai & Downie (2005) found that the cement gland was present and functional in three species of foam-nesting leptodactylids (*Physalaemus pustulosus*, *Leptodactylus validus* and *L. bolivianus*) but absent in the fourth, *Leptodactylus fuscus* and in the direct-developing *Eleutherodactylus urichi*. Absence in the direct developer was expected, but absence in *L. fuscus* was more surprising. *Leptodactylus fuscus* deposits eggs in a mass of foam in a burrow near a temporary pool site. After hatching, the larvae can remain in the foam nest and produce a new kind of foam which replaces the original nest foam. If heavy rains do not fall, the larvae remain in the nest in a state of arrested development for up to several weeks until rain eventually washes them into the nearby pool, where the larvae begin to feed (Downie, 1984, 1994). Nokhbatolfighahai & Downie (2005) suggested that the absence of cement glands in *L. fuscus* may be because it is obligatory for *L. fuscus* larvae to remain in the nest until past the phase when the cement gland is functional in other species, i.e. until the stage of independent feeding. The work reported here is an exploration of this hypothesis, using a comparison of reproductive strategies amongst three foam-nesting species exhibiting different reproductive modes.

MATERIALS AND METHODS

Spawn of three leptodactylid species (*Leptodactylus fuscus*, *L. validus* and *Physalaemus pustulosus*) was collected from various locations in Trinidad, West Indies, over several field seasons. Spawn characteristics are described by Kenny (1969). Spawning sites were identified from adult calls at night and spawn collected

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the next morning. *Leptodactylus fuscus* spawn is hidden in burrows on the banks of temporary pools, and we located burrows by probing with a metal spoon handle. *Leptodactylus validus* spawn is located at the edges of pools, usually in forest, and hidden by dead leaves: we located spawn by systematic searching of the margins of pools. *Physalaemus pustulosus* spawn is easily located floating on the surface of pools in the open. After collection, spawns were incubated in a laboratory at 27-28°C air temperature either floating on the surface of dechlorinated tap water (*L. validus*, *P. pustulosus*) or in a closed container on the surface of damp tissue paper (*L. fuscus*) until appropriate stages were reached (Gosner, 1960, staging used for all species). Clutch sizes were counted from the number of hatchlings, and also by checking foam nests for undeveloped eggs. Specimens for microscopic examination were fixed in buffered neutral formalin or 2.5% glutaraldehyde in phosphate buffer. Measurements of fixed embryos were made using a dissecting microscope with calibrated eyepiece graticle, and drawings were made with the aid of a drawing tube.

To test how well embryos developed if they entered water at an earlier stage than normal, eggs were carefully removed singly, using forceps, from several foam nests and floated on the surface of dechlorinated, aerated tapwater in the laboratory. For *L. fuscus*, embryos were also removed from their foam nests and placed in groups of 10 on the surface of damp tissue in 9 cm diameter Petri dishes. Success of development was monitored daily over several days.

RESULTS

Tables 1 and 2 show reproductive and developmental data for the three species, while Fig. 1 shows camera lucida drawings of embryo and larval morphology up to stage 25-26 and measurements of relative tail length. *Leptodactylus fuscus* produces small numbers of large eggs; *L. validus* large numbers of small eggs; *P. pustulosus* is intermediate. Correcting for body size, *L. validus* puts the most resource into each clutch (Table 1), *L. fuscus* the least (assuming that resource input per egg is proportional to egg volume). *L. validus* and *P.*

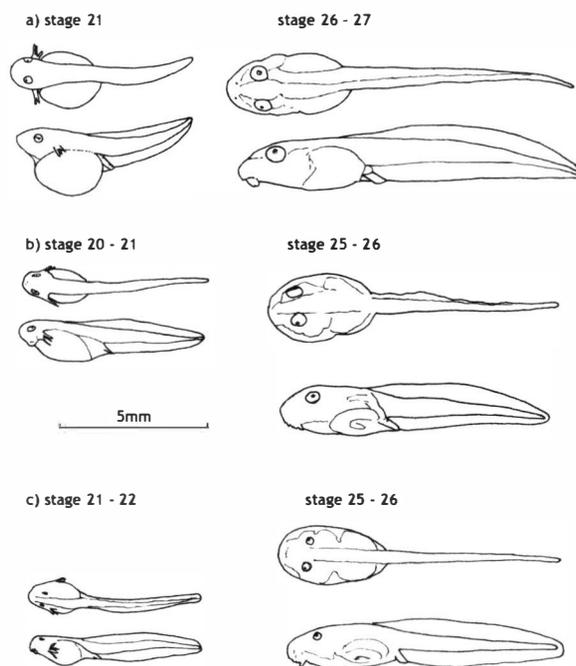


FIG. 1. Camera lucida drawings comparing early larval morphology of (a) *Leptodactylus fuscus*, (b) *Physalaemus pustulosus* and (c) *Leptodactylus validus*. Drawings made at overall magnification of $\times 9.4$. Each stage shown from dorsal and lateral aspects. All from formol-saline fixed specimens. Ratio of tail length (from middle of vent) to total body length, based on mean of three specimens is: *L. fuscus* stage 21, 50.2%; stage 26-27, 59.7%. *P. pustulosus* stage 21, 54.9%; stage 25-26 58.3%. *L. validus* stage 21-22 62.7%; stage 25-26, 59.0%.

pustulosus hatch at about the same stage and time since deposition, and leave the nest for the water below soon after (Downie, 1993 showed that some *P. pustulosus* remain in the nest for up to about 20 hr after hatching, but others leave much sooner. Comparable observations have not been made on *L. validus*). By the time they leave the nest, the larvae of these two species have greatly reduced yolk sacs and well developed tails. *Physalaemus pustulosus* larvae attach by their cement glands to the sides or bottom of their container; *L. validus* mainly hang from the lower side of their foam nest.

TABLE 1. Reproductive data for three leptodactylids. Female snout-vent length (SVL) from Murphy (1997). Egg volume calculated from diameter using formula for a sphere. 1, clutch sizes counted to nearest 100. Size related reproductive output is mean number of eggs per clutch times egg volume divided by female SVL.

Species and female SVL	Clutch size mean \pm SD, <i>n</i>	Egg diameter (mm): mean \pm SD, <i>n</i>	Mean egg volume	Size related reproductive output
<i>Leptodactylus fuscus</i> 50 mm	154 \pm 38 <i>n</i> =10	1.97 \pm 0.17 <i>n</i> =9	4.0	12.3
<i>Physalaemus pustulosus</i> 32 mm	420 \pm 135 <i>n</i> =13	1.51 \pm 0.06 <i>n</i> =11	1.8	23.6
<i>Leptodactylus validus</i> 51 mm	1500 \pm 400 ¹ <i>n</i> =9	1.36 \pm 0.07 <i>n</i> =11	1.3	38.2

TABLE 2. Developmental data for three leptodactylids. All species incubated at 27-28°C. 1, timing assumes eggs were fertilized in the middle of the night prior to the morning of collection.

Species and stage of hatching	Time to hatching (days) ¹	Time to stage 25 (days) ¹	Total length, mm (mean±SD, n) at different stages		
			22	23	25
<i>Leptodactylus fuscus</i> stage 18-19	2.5	4.5	5.7±0.1 n=7	6.8±0.2 n=7	8.4±0.2 n=9
<i>Physalaemus pustulosus</i> stage 20-21	2.0	3.5	6.8±0.3 n=4	7.6±0.2 n=8	8.3±0.2 n=6
<i>Leptodactylus validus</i> stage 20-21	2.5	3.5	5.9±0.3 n=8	-	7.3±0.7 n=8

In contrast, *L. fuscus* hatches slightly earlier in terms of developmental stage than the other two species; at comparable stages up to stage 25, they have larger yolk sacs and more poorly developed tails. Overall, development to the stage of independent feeding takes longer than in the other two species. Body length at stage 22 is shorter in *L. fuscus* than the other two species, but longest by stage 25.

Table 3 shows the results of experiments where *L. fuscus* embryos were transferred singly either to the surface of water or to damp tissue at different developmental stages. In neither case was development very successful until about stage 22 (day 3, when embryos were already hatched). Mortality was high in both cases for embryos transferred during the first two days of development. However, the pattern of mortality was different in the two cases. Early embryos transferred to the water surface floated until hatching, then sank to the bottom. Many failed to reach hatching stage and those that did hatch often appeared malformed and soon died. Early embryos transferred to damp tissue tended to develop successfully past the hatching stage and died between days 3 and 4: it looked as if the lack of support for the large yolk mass when the embryo was outside the foam nest had some kind of harmful effect, possibly blocking the yolk sac circulation. Embryos transferred to the damp tissue surface at a later stage survived and developed well.

When a similar experiment was carried out on *P. pustulosus*, all embryos either on water or tissue surface developed normally to hatching. Those in water all continued to develop normally; those on damp tissue died soon after hatching. No *L. validus* embryos were available for comparable experiments.

DISCUSSION

Our conclusion from the observations reported here is that *P. pustulosus* develops like most anurans, except in a floating foam nest. Once hatched, with a reduced yolk mass, larvae enter water and attach to a surface by their cement gland until the external gills are resorbed and feeding can begin. Tails are well developed at an early stage to allow active swimming. Out of water, hatchlings have very brief survival capacity, a feature noted by Downie (1988) and also for later stage tadpoles by Downie & Smith (2003). We expect that *L. validus* hatchlings would also show poor survival out of water, but have not tested them. *Leptodactylus fuscus* shows several differences from the *P. pustulosus* reproductive mode: the foam nest is in a terrestrial burrow, and eggs are both larger and in relatively smaller numbers. Development to the stage of independent feeding takes longer and the tail is relatively poorly developed at early stages. Downie (1994) has shown that after hatching, *L. fuscus* larvae make a foam which replaces the original nest foam and can remain in the nest for up to several

TABLE 3. Survival of *L. fuscus* and *P. pustulosus* eggs transferred at different stages to the surface of water or damp tissue paper.

Stage on transfer	Percentage of survival to stage 26 (<i>L. fuscus</i> , day 6; <i>P. pustulosus</i> , day 4); n=number of eggs at start; <i>L. fuscus</i> pooled from several clutches, <i>P. pustulosus</i> from one clutch.	
	Transfer to water	Transfer to tissue
<i>L. fuscus</i>		
Cleavage (day 0)	0 (n=12)	10.0 (n=20)
Gastrula (day 1)	39.6 (n=48)	33.3 (n=30)
Hatching (day 2)	56.0 (n=50)	0 (n=34)
Stage 22 (day 3)	100.0 (n=50)	50.0 (n=10)
Stage 23 (day 4)	100.0 (n=31)	100.0 (n=10)
<i>P. pustulosus</i>		
Cleavage (day 0)	100.0 (n=20)	0 (n=20)

weeks, depending on rainfall. The results reported here suggest that at least a brief post-hatching period in the foam nest is necessary for effective development. Early entry to water leads to high mortality and early hatchlings do not survive well on a damp tissue surface, though they do at a somewhat later stage (Downie & Smith, 2003). Poor early survival either in water or on damp tissue may be related to the large size of the yolk mass and the relatively poorly developed locomotory organs. To improve survival chances, the larvae remain in the foam nest till the yolk mass is reduced and the tail fully developed for locomotion. This takes the larvae beyond the stage when a cement gland is normally functional. As has been repeatedly demonstrated, natural selection leads to the reduction and eventual deletion of functionless organs (Fong *et al.*, 1995). Nokhbatol-foghahai & Downie's (2005) finding of vestiges of cement gland development in *L. fuscus* suggests that this deletion is fairly recent. We would expect the cement gland to be lacking in other leptodactylids that spend a post-hatching period in the nest, even if they have a tadpole stage, such as other species in the *Leptodactylus* 'fuscus' group (Heyer, 1969).

It is not clear why *L. fuscus* should hatch relatively early, if the embryos must then spend a further period in the nest. In other frogs with large eggs and later emergence to water, such as the myobatrachid *Pseudophryne bibroni* (Bradford & Seymour, 1985) or members of the genus *Phyllomedusa* (Pyburn, 1980), hatching is after stage 25. A consequence of early hatching in *L. fuscus* is the breakdown of the original nest foam (Downie, unpublished observations) which is then replaced by the new foam made by the larvae (Downie, 1984). However, it is not obvious that this is an advantage, unless it is easier for the larvae to extricate themselves from the new foam, once the nests are inundated, than it would be from the original foam. Another possibility is that hatching allows the larvae to escape from nest predators such as frogfly maggots (Downie *et al.*, 1995), but their poor motility immediately after hatching makes this unlikely.

Heyer (1969) outlined a possible adaptive scenario for the reproductive modes found in the genus *Leptodactylus*. Floating foam nests, as found in the 'melanonotus' and 'ocellatus' species groups evolved as a means of avoiding aquatic predators and the desiccation of temporary pools; nests in potholes near water in the 'pentadactylus' group gave further protection from aquatic predators; nests in terrestrial burrows such as in the 'fuscus' group, removed the linkage of reproduction and rainfall and gave hatchlings a head-start over competitor species; finally, in the 'marmoratus' group, later moved to the genus *Adenomera* (Heyer, 1974), large eggs developed through to metamorphosis in a terrestrial egg chamber, removing the link to the aquatic habitat. More recently, Prado *et al.* (2002) expanded Heyer's list by two extra modes: nest hole excavation in *L. podicipinus* and larval development in the foam nest within a burrow in some species of the

'pentadactylus' group: Prado *et al.*'s conjecture that the latter mode might involve an extra source of nutrition, has been confirmed by Gibson & Buley (2004) for *L. fallax* where the female provides repeated batches of trophic eggs. We expect that *L. fallax* will lack the cement gland.

The question remains whether or not these different modes represent a single trend towards terrestrial development. Heyer (1969) considered that the 'fuscus' and 'marmoratus' groups represented two separate shifts from an original aquatic foam nest. In our view, more detailed work of the kind we report needs to be done to tease out the fine details of the changes involved in these various lineages. There are several differences between the reproductive strategy and associated features of *L. fuscus* and the two floating foam nesting species we have used for comparison: these include egg size, egg number, reproductive effort, egg location, developmental time out of water, survival and cement gland presence. An additional factor requiring investigation is the inter-clutch interval for the females of foam-nesting species. Davidson & Hough (1969) found that females of *Physalaemus* (= *Engystomops*) *pustulosus*, could ovulate every 4-5 weeks in a laboratory population, but equivalent field data are lacking. It is not clear which of these differences are likely to have been primary, and which are consequences of the shift to terrestrial reproduction. A robust phylogeny of the leptodactylids would be a great help in attempting to explain these evolutionary changes. In addition, we need reproductive strategy data from more species, including the state of the cement gland.

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MOLECULAR PHYLOGENY OF BRAZILIAN *MABUYA* (REPTILIA, SQUAMATA, SCINCIDAE) OF THE *AGILIS/CAISSARA/HEATHI* COMPLEX

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Phylogenetic relationships among neotropical skinks of the genus *Mabuya* are currently unknown. Three species, *Mabuya agilis*, *M. caissara* and *M. heathi* are morphologically very similar and appear to be closely related, but their relationships have never been studied. Here we examine their phylogenetic relationships using partial sequences of the mitochondrial 12S and 16S rRNA genes. Nine populations of *M. agilis*, four of *M. heathi* and one of *M. caissara* were sampled, as well as one population from four other South American *Mabuya* species. Results of both maximum likelihood and maximum parsimony analyses reveal a strongly supported monophyletic group comprising all populations of *agilis*, *caissara* and *heathi*, but relationships of this clade with the other *Mabuya* species were not resolved. Genetic distances among members of the *agilis/caissara/heathi* clade ranged from 0.0% to 2.6%, whereas distances between its members and the other four congeners ranged from 6.0% to 8.6%. Genetic distances and the internal tree structure of the *agilis/caissara/heathi* clade are somehow consistent with the geographic location of the sampled populations. Based on our results, we suggest that this complex may represent a single species, though more data are needed to verify this.

Key words: Brazil, lizard, mitochondrial DNA, phylogenetics, skink

INTRODUCTION

Intercontinental relationships within the circumtropical genus “*Mabuya*” Fitzinger (Scincidae: Lygosominae), are far more complex than previously thought (Mausfeld *et al.*, 2002; Carranza & Arnold, 2003). A molecular analysis of the genus recently carried out by Mausfeld *et al.* (2002) demonstrated that “*Mabuya*” consists of four separated and deep evolutionary lineages, representing distinct and well-supported monophyletic radiations. To reflect the independent origins of the American, Asian, Afro-Malagasy and Cape Verdian groups, the genus “*Mabuya*” has been partitioned into four genera, keeping the name *Mabuya* for the neotropical species.

However, the systematics and taxonomy of neotropical *Mabuya* are still in an unstable state and remain poorly understood. Since Dunn’s (1935) work, no other comprehensive revision of New World *Mabuya* has been carried out. Several subsequent contributions have added to or modified Dunn’s original taxonomic arrangement, including the description of new species, the revalidation of old names previously in the synonymy of *M. mabouya* (see Rodrigues, 2000 for a review), the raising of a subspecies to specific status (Mausfeld & Lötters, 2001) and the restriction of the name *M. mabouya* to populations of the southern Lesser Antilles (Miralles, 2005). Nevertheless, the situation is still far from satisfactory. Indeed, even the recently proposed taxonomic arrangement of Avila-Pires (1995) concerning the two most common Amazonian species has not been universally accepted

(see Rodrigues, 2000). Currently, the genus *Mabuya* is represented in continental South America by 17 species, most of which occur in Brazil (Rodrigues, 2000; Mausfeld & Lötters, 2001; Miralles *et al.*, 2005), but the phylogenetic relationships among them remain unstudied.

Recently, Rodrigues (2000) divided the South American *Mabuya* species into four phenetic groups, but cautioned that such groups were ‘only convenient and based on similarity’. One of those groups comprised ‘small species with normal (i.e. not prominent or acuminate) snout, paired frontoparietals and vertebral stripes on body’ and included five species: *M. dorsivittata*, *M. guaporicola*, *M. heathi*, *M. agilis* and *M. caissara*. The former two (which Dunn [1935] believed to be closely related to each other) are rather distinctive in colour pattern and in some characteristics of scalation and morphology (*M. dorsivittata* normally has three supraoculars, instead of four as in the remaining species; *M. guaporicola* has comparatively short limbs and a high number of presacral vertebrae [Dunn, 1935; Greer *et al.*, 2000]), and do not occur in coastal areas. The latter three species, which are common in open habitats along the Atlantic rainforest biome of eastern Brazil (though *M. heathi* also occurs throughout the semi-arid caatingas), are very similar to each other. They all share the same general colour pattern, with a dark lateral band (extending from the snout to the tip of the tail) bordered below by a vivid white stripe that extends from the lower lip to the groin, and a mid-dorsal stripe with irregular dark borders (which can also be described as a pair of parallel irregular dark stripes, as in Schmidt & Inger’s [1951]) description of *M. heathi*), which is usually

TABLE 1. List of voucher specimens for each species included in the present study, with their respective localities, collection numbers and accession numbers (12S, 16S). All samples are from Brazil, except those of *Eumeces obsoletus* and *Mabuya* sp. Brazilian state codes are: BA - Bahia; CE - Ceará; DF - Distrito Federal; ES - Espírito Santo; RJ - Rio de Janeiro; RN - Rio Grande do Norte; SP - São Paulo. Acronyms: CHUNB - Coleção Herpetológica da Universidade de Brasília; MNRJ - Museu Nacional, Rio de Janeiro; UFC - Universidade Federal do Ceará; ZFMK - Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

Species	Locality	Collection number	Accession number
<i>Eumeces obsoletus</i>	USA	ZFMK 77248	AF548781, AF549169
<i>Mabuya</i> sp.	Tobago	ZFMK 62603	AY070339, AY070357
<i>M. dorsivittata</i>	Cunha, SP	MNRJ 9338	AY070346, AY070363
<i>M. macrorhyncha</i>	Queimada Grande island, SP	MNRJ 7287	AF548782, AF549170
<i>M. nigropunctata</i>	Brasília, DF	CHUNB 9624	AF548783, AF549171
<i>M. heathi</i>	Crateús (Serra das Almas), CE	UFC L2288	AY070330, AY070349
<i>M. heathi</i>	Natal, RN	MNRJ 8361	AF548784, AF549172
<i>M. heathi</i>	Abrolhos archipelago (Siriba island), BA	MNRJ 6663	AF548786, AF549174
<i>M. heathi</i>	Abrolhos archipelago (Sueste island), BA	MNRJ 6655	AF548785, AF549173
<i>M. agilis</i>	Prado, BA	MNRJ 9337	AY070326, AY070347
<i>M. agilis</i>	Guriri, ES	MNRJ 9543	AF548790, AF549178
<i>M. agilis</i>	Setiba, ES	MNRJ 9524	AF548791, AF549179
<i>M. agilis</i>	Praia das Neves, ES	MNRJ 9514	AF548789, AF549177
<i>M. agilis</i>	Carapebus (Jurubatiba restinga), RJ	MNRJ 9508	AF548792, AF549180
<i>M. agilis</i>	Massambaba, RJ	MNRJ 9560	AF548794, AF549182
<i>M. agilis</i>	Maricá (Barra de Maricá restinga), RJ	MNRJ 9561	AF548796, AF549184
<i>M. agilis</i>	Rio de Janeiro (Grumari beach), RJ	MNRJ 9491	AF548795, AF549183
<i>M. agilis</i>	Ilha Grande, Angra dos Reis, RJ	MNRJ 9494	AF548793, AF549181
<i>M. caissara</i> 1	Caraguatatuba (Massaguassu beach), SP	MNRJ 9476	AF548787, AF549175
<i>M. caissara</i> 2	Caraguatatuba (Massaguassu beach), SP	MNRJ 9485	AF548788, AF549176

lighter in colour than the rest of the metallic-brown dorsum. Such characters are mentioned, in varying detail, in the descriptions of each of the three species (Raddi, 1823; Schmidt & Inger, 1951; Rebouças-Spieker, 1974). Rodrigues (1990) implicitly suggested that *M. agilis* and *M. heathi* are conspecific and closely related to *M. caissara*, but did not comment the subject any further. Rocha (2000) also suggested that *M. caissara* and *M. agilis* should be closely related phylogenetically, given their strong morphological similarity. The geographic distributions of the three species appear to be contiguous in eastern Brazil: *Mabuya heathi* ranges from Fortaleza, in Ceará state, across the semi-arid "caatinga" habitats (see Eiten, 1992) of north-eastern Brazil and along the coast, south to Salvador, in Bahia state (Vanzolini *et al.* 1980; Peters & Donoso-Barros 1986), with an insular population recently reported from the Abrolhos archipelago (Dutra & Vrcibradic, 1998); *Mabuya agilis* occurs along the coastal lowlands and slopes of adjacent mountain ranges, from southern Bahia state to Rio de Janeiro state (Vanzolini, 1988; Rocha, 2000; Rocha *et al.*, 2002a); *M. caissara* is apparently restricted to the northern coast of São Paulo state, from Ubatuba to Bertioga (Rebouças-Spieker, 1974; Vanzolini, 1988).

In the present study we examine the relationships among *Mabuya agilis*, *M. caissara* and *M. heathi*, based on an analysis of mtDNA sequences, to assess if they are reciprocally monophyletic. We include four other neotropical *Mabuya* species in the analysis in order to

assess whether those three species form a monophyletic group.

MATERIALS AND METHODS

To examine the genetic variation within and among *Mabuya agilis*, *M. caissara* and *M. heathi*, liver or muscle tissue samples were collected from nine populations of *M. agilis*, one population of *M. caissara*, and four populations of *M. heathi* (Table 1). Among them are samples from the type localities of *M. agilis* (Rio de Janeiro municipality) and of *M. caissara* (Massaguassu beach, Caraguatatuba). We could not obtain samples from the type locality of *M. heathi* (Fortaleza, Ceará state), but we got material from an inland locality (Crateús) within the semi-arid "caatinga" domain in Ceará state, about 340 km SW of Fortaleza (which is a coastal locality). We sampled one individual for each population except for *M. caissara* from Caraguatatuba, in which case samples from two individuals were used (Table 1). Sampled localities for specimens of the *agilis/caissara/heathi* complex used in this study are depicted in Fig. 1.

Tissue samples of four other neotropical congeners, *M. dorsivittata*, *M. macrorhyncha*, *M. nigropunctata* and *Mabuya* sp. (a specimen from Tobago; the taxonomic status of *Mabuya* populations from that island requires confirmation [A. Miralles, pers. comm.]) were also included in the analysis (Table 1). The latter three taxa were once part of Dunn's (1935) *M. m. mabouya* together with members of the *agilis/caissara/heathi*

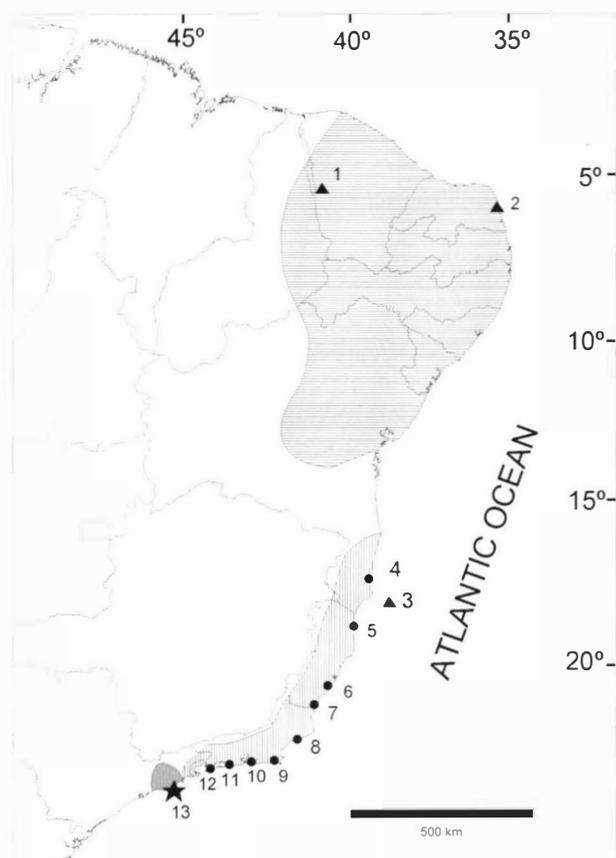


FIG. 1. Map of eastern Brazil showing the localities from which samples of *Mabuya heathi* (triangles), *M. agilis* (dots) and *M. caissara* (star) used in this study were obtained: 1 - Crateús, CE; 2 - Natal, RN; 3 - Abrolhos archipelago, BA; 4 - Prado, BA; 5 - Guriri, ES; 6 - Setiba, ES; 7 - Praia das Neves, ES; 8 - Carapebus, RJ; 9 - Massambaba, RJ; 10 - Maricá, RJ; 11 - Grumari, RJ; 12 - Ilha Grande, RJ; 13 - Caraguatatuba, SP. Brazilian state codes are: BA - Bahia; CE - Ceará; ES - Espírito Santo, RJ - Rio de Janeiro; RN - Rio Grande do Norte; SP - São Paulo. Approximate geographic distributions for each species, given as horizontal stippling (*M. heathi*), vertical stippling (*M. agilis*) and shading (*M. caissara*), are based on the literature and on data from material deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

complex, whereas *M. dorsivittata* was included by Rodrigues (2000) in a phenetic group that also contained *M. agilis*, *M. caissara*, *M. heathi* and *M. guaporicola* (unfortunately, we could not obtain tissue samples from this last species). The North American scincine *Eumeces obsoletus* was used as outgroup.

Individuals from populations of the *agilis/caissara/heathi* complex included in our study are virtually indistinguishable in general appearance, except for *M. heathi* from the Abrolhos archipelago. The latter differ from members of other populations of the complex in being slightly lighter in coloration, with a caramel-brown to tan (rather than bronze) dorsum and a brown (rather than black) lateral dark band. Individuals from Praia das Neves and Setiba show some tendency towards larger body size than those from the other localities, but are otherwise identical to them (see Rocha *et al.*, 2002a). All voucher specimens used in the present study are

listed in Table 1, with their collection locality, voucher numbers, and GenBank accession numbers.

DNA was extracted from the tissue samples using QiaAmp tissue extraction kits (Qiagen). We used the primers 16sar-L (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16sbr-H (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') of Palumbi *et al.* (1991) to amplify a section of the mitochondrial 16S ribosomal RNA gene. PCR cycling procedure was as follows. Initial denaturation step: 90 s at 94°C; 33 cycles: denaturation 45 s at 94°C, primer annealing for 45 s at 55°C, extension for 90 s at 72°C. Additionally, we used the primers 12SA-L (light chain; 5' - AAA CTG GGA TTA GAT ACC CCA CTA T - 3') and 12SB-H (heavy chain; 5' - GAG GGT GAC GGG CGG TGT GT - 3') of Kocher *et al.* (1989) to amplify a section of the mitochondrial 12S ribosomal RNA gene. Cycling procedure was as follows: 35 cycles: denaturation 45 s at 94°C, primer annealing for 60 s at 50°C, extension for 120 s at 74°C (12S).

PCR products were purified using Qiaquick purification kits (Qiagen). Sequences were obtained using an automatic sequencer (ABI 377). The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 550 bp (16S), and 398 bp (12S). Sequences have been submitted to GenBank (Table 1).

Sequences were aligned using the computer program ClustalX (Thompson *et al.*, 1997; default parameters). Alignment was subsequently adjusted manually using the computer program Se-Al 1.0a1 (Rambaut, 1996). We explored the quality of our alignment by varying alignment gap opening cost (6, 9, 12) and comparing between all three different alignments. In the 12S data set no ambiguous sites could be detected, in the 16S data set one ambiguously aligned region of 12 bp was found; these sites were excluded from the analysis (Gatesy *et al.*, 1993; Milinkovitch & Lyons-Weiler, 1998). The complete alignment is available from the authors on request.

Confidence in the phylogenetic signal for the data-set was assessed using two different methods implemented in PAUP*4.0b8 (Swofford, 2002). The presence of a significant phylogenetic signal was estimated using the *g*1 statistic (Hillis & Huelsenbeck, 1992) estimated from 100,000 randomly generated parsimony trees (excluding the outgroup), and the permutation-tailed-probability (PTP) test (heuristic search with random sequence addition and 10 replicates; randomized ingroup taxa only) as suggested by Faith & Cranston (1991), with 100 replicates.

Prior to phylogenetic reconstruction, we tested for homogeneity of base frequencies among taxa using the χ^2 test as implemented in PAUP*4.0b8 (which ignores correlation due to phylogenetic structure): (1) over all sites, (2) over parsimony-informative sites only, (3) without constant sites (parsimony-uninformative and constant sites will mislead the χ^2 test (Misof *et al.*,

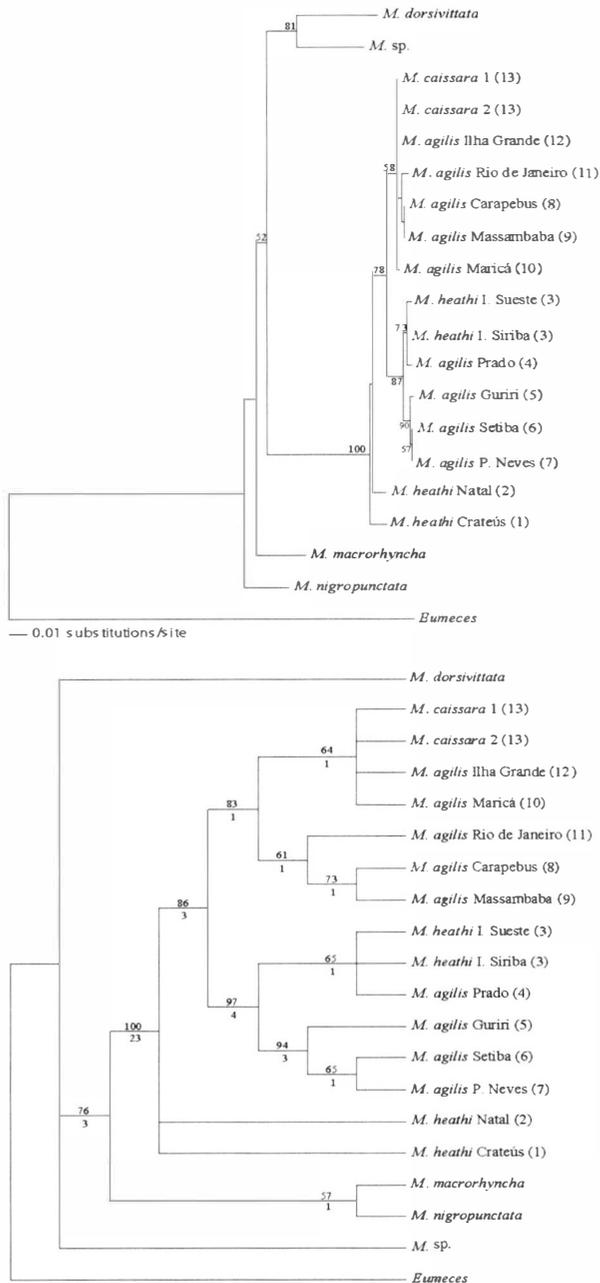


FIG. 2. Phylogram of the maximum likelihood tree (top) and the cladogram of the strict-consensus maximum parsimony tree (bottom) obtained from PAUP* searches using *Eumeces obsoletus* as outgroup. Numbers above nodes represent bootstrap proportions for 2000 and 100 pseudoreplicates for parsimony and likelihood analyses, respectively. Bootstrap proportions less than 50% are not shown. Numbers below nodes in MP tree represent decay indices.

2001). All phylogenetic reconstructions were conducted on alignment unambiguous characters only, with the combined data set of the 16S and 12S gene fragments, and were performed with PAUP*4.0b8 (Swofford, 2002). We performed maximum-parsimony (MP) and maximum likelihood (ML) reconstructions. Parsimony reconstructions were performed with heuristic searches on parsimony-informative characters only, with 10 random stepwise addition replicates, and tree bisection-reconnection (TBR) branch-swapping. Gaps were treated as a 5th character state (Giribet & Wheeler, 1999; Simmons & Ochoterena, 2000; Simmons *et al.*,

2001). When more than a single tree was found, a strict consensus tree was generated.

MODELTEST 3.06 (Posada & Crandall, 1998, 2001) was used to select the best-fit model for nucleotide substitution for our data set. Parameters of the model (substitution parameters, shape of gamma distribution, proportion of invariable sites) were estimated from the data set, without sites containing gaps (Aguinaldo *et al.*, 1997), using a neighbour-joining starting-tree with p-distance. The ML tree was calculated with the parameter estimates obtained under the best-fit model. A heuristic search was made with 10 replicates of random stepwise addition and tree bisection-connection (TBR) branch swapping. A matrix of pairwise sequence differences for the combined 16S and 12S rRNA genes was calculated using the p-distance.

The relative branch support of the phylogenetic analysis was evaluated with 2000 bootstrap pseudoreplicates (heuristic search, with 10 replicates of random stepwise addition, TBR branch-swapping, parsimony-informative characters only) for MP, and 100 replicates for ML analysis (heuristic search, 10 replicates of random stepwise addition, TBR branch-swapping). As an alternative measure of nodal support, decay indices (Bremer, 1994) were calculated by running heuristic searches (100 random addition replicates, with TBR branch-swapping, and saving trees one step longer in each run) using TreeRot, version 2 (Sorenson, 1999) and PAUP*4.0b8.

RESULTS

The analyzed sequences from the 16S and 12S rRNA genes constitute a matrix of 936 characters. Of the 936 analyzed character sites analyzed, 117 sites were variable, and 88 were parsimony-informative. The matrix for the absolute number of DNA-sequence differences and uncorrected p-distances for all nucleotide sites is presented in Table 2. Phylogenetic signal is clearly present in the data set ($gI = 1.2306$, $P = 0.01$; PTP test, $P = 0.01$). When all characters were included, we found no significant deviation from the homogeneity of base frequencies among taxa ($\chi^2 = 6.710$, $P = 1.00$, $df = 57$). The same was true for the parsimony-informative sites only ($\chi^2 = 25.167$, $P = 0.9999$, $df = 57$) and without constant sites ($\chi^2 = 35.568$, $P = 0.988$, $df = 57$).

The MP analysis produced eight equally most-parsimonious trees (tree length = 191; CI = 0.639; RI = 0.766, RC = 0.489). The comparison between the different likelihood scores for each model showed that the TrN + I + G model (Tamura & Nei, 1993) was determined to be the appropriate model for our data set. This model incorporates unequal base frequencies [$\pi_{(A)} = 0.339$, $\pi_{(T)} = 0.219$, $\pi_{(C)} = 0.255$, $\pi_{(G)} = 0.187$, a proportion of invariable sites ($I = 0.5431$), and a gamma distribution shape parameter ($G = 0.523$).

Both the strict consensus of the optimal MP trees and the best ML tree are shown in Fig. 2. Both MP and ML methods produced very similar topologies (with a few

TABLE 2. Summary of the absolute number of DNA-sequence differences (above the diagonal) and uncorrected p-distances (below the diagonal)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 <i>Eumeces</i>		128	135	133	128	154	146	150	149	150	148	148	148	149	151	152	152	152	149
2 <i>Mabuya</i> sp.	0.143		52	54	54	65	64	67	67	65	66	66	66	65	68	64	67	67	65
3 <i>M. dorsivittata</i>	0.151	0.058		58	59	73	68	77	52	74	75	75	77	73	78	76	75	75	73
4 <i>M. nigropunctata</i>	0.149	0.060	0.064		40	58	62	58	59	63	61	61	63	64	59	58	59	59	64
5 <i>M. macrorhyncha</i>	0.142	0.060	0.065	0.043		58	56	58	59	59	57	57	60	58	59	57	58	58	58
6 <i>M. heathi</i> Crateús	0.172	0.072	0.081	0.062	0.062		15	21	23	17	21	21	21	18	21	24	23	23	18
7 <i>M. heathi</i> Natal	0.163	0.071	0.075	0.067	0.060	0.016		22	24	17	19	19	17	16	22	21	22	22	16
8 <i>M. heathi</i> I. Siriba	0.167	0.075	0.085	0.063	0.062	0.023	0.024		2	15	15	15	15	14	2	7	6	6	14
9 <i>M. heathi</i> I. Sueste	0.166	0.075	0.085	0.064	0.064	0.025	0.026	0.002		17	17	17	17	16	4	9	8	8	16
10 <i>M. agilis</i> Maricá	0.167	0.072	0.082	0.068	0.064	0.018	0.018	0.016	0.018		4	4	6	1	15	18	17	17	1
11 <i>M. agilis</i> Massambaba	0.165	0.073	0.083	0.066	0.061	0.023	0.020	0.016	0.018	0.004		0	4	3	15	18	17	17	3
12 <i>M. agilis</i> Carapebus	0.165	0.073	0.083	0.066	0.061	0.023	0.020	0.016	0.018	0.004	0.000		4	3	15	18	17	17	3
13 <i>M. agilis</i> Rio de Janeiro	0.165	0.073	0.085	0.068	0.065	0.023	0.018	0.016	0.018	0.007	0.004	0.004		5	15	18	17	17	5
14 <i>M. agilis</i> Ilha Grande	0.166	0.072	0.081	0.069	0.062	0.019	0.017	0.015	0.017	0.001	0.003	0.003	0.005		14	17	16	16	0
15 <i>M. agilis</i> Prado	0.168	0.076	0.086	0.064	0.064	0.023	0.024	0.002	0.004	0.016	0.016	0.016	0.016	0.015		9	8	8	14
16 <i>M. agilis</i> Guriri	0.169	0.071	0.084	0.062	0.061	0.026	0.023	0.008	0.010	0.019	0.019	0.019	0.019	0.018	0.010		3	3	17
17 <i>M. agilis</i> P. Neves	0.169	0.075	0.083	0.064	0.062	0.025	0.024	0.007	0.009	0.018	0.018	0.018	0.018	0.017	0.009	0.003		0	16
18 <i>M. agilis</i> Setiba	0.169	0.075	0.083	0.064	0.062	0.025	0.024	0.007	0.009	0.018	0.018	0.018	0.018	0.017	0.009	0.003	0.000		16
19 <i>M. caissara</i>	0.166	0.072	0.081	0.069	0.062	0.019	0.017	0.015	0.017	0.001	0.003	0.003	0.005	0.000	0.015	0.018	0.017	0.017	

minor differences regarding *Mabuya* species outside the *agilis/caissara/heathi* complex). Both trees show strong bootstrap support (100%) for a monophyletic group containing all *Mabuya agilis*, *M. caissara* and *M. heathi*. Within this group, however, *M. agilis* and *M. heathi* were not recovered as monophyletic by neither MP nor ML analyses. Genetic differences within the *agilis/caissara/heathi* clade varied from 0% (between the two *M. caissara* samples, between *M. caissara* and *M. agilis* from Ilha Grande, between *M. agilis* from Massambaba and Carapebus, and between *M. agilis* from Setiba and Praia das Neves) to 2.6% (between *M. heathi* from Crateús and *M. agilis* from Guriri, and between individuals of *M. heathi* from Natal and Sueste island, Abrolhos) (Table 2). The lowest genetic difference between a member of the *agilis/caissara/heathi* group and any other *Mabuya* species was 6.0% (between *M. heathi* from Natal and *M. macrorhyncha*) (Table 2). The *agilis/caissara/heathi* clade itself was found (in both MP and ML analyses) to consist of a southern *agilis/caissara* group (populations from the states of Rio de Janeiro and São Paulo; this cluster was, however, only weakly supported in the ML tree), a northern *agilis/heathi* group (populations from the states of Bahia and Espírito Santo), and the two northernmost *heathi* specimens from Natal and Crateús (Fig. 2). These latter two *M. heathi* samples were sister taxa to the clade formed by the southern and northern groups in the ML tree, whereas in the MP tree they form a basal polytomy (Fig. 2).

The relationships of the *agilis/caissara/heathi* clade with respect to the other neotropical *Mabuya* sampled for this study were not resolved. In the ML tree (see Fig. 2) a moderately supported (81% bootstrap) *M. dorsivittata*-*M. sp.* clade is sister to the *M. agilis/caissara/heathi* clade, although the bootstrap support is very low. In the MP analysis (see Fig. 2), a weakly supported (57% bootstrap) *M. nigropunctata*-*M. macrorhyncha* clade appears as sister to the *agilis/caissara/heathi* complex with moderate (76%) bootstrap support (Fig. 2).

DISCUSSION

The *Mabuya agilis/caissara/heathi* complex forms, in both MP and ML analyses, a well-supported monophyletic group with rather small genetic distances among its members (0.0-2.6%). The clade contains two well-defined sub-groups: a southern *M. agilis/caissara* group, and a northern *M. agilis/heathi* group, though the former has only weak bootstrap support (58%) in the ML tree. Basal to these two sub-groups are the two northernmost *M. heathi* specimens. Non-monophyly of *M. agilis* is indicated by the fact that some of its populations are closely related to *M. caissara* whereas others are closely related to *M. heathi*. Non-monophyly of *M. heathi* is revealed by the fact that the insular populations are closely related to northern *M. agilis* populations whereas other populations are more distantly related to all other members of the complex.

Even though our results do not allow recognition of the sister group to the *agilis/caissara/heathi* clade (due to the lack of a high bootstrap support for the respective node), they definitely show that *M. dorsivittata*, *M. sp.*, *M. macrorhyncha* and *M. nigropunctata* are all substantially divergent from it. Although Rodrigues (2000) included *M. dorsivittata*, *M. agilis*, *M. caissara* and *M. heathi* in the same phenetic group (together with *M. guaporicola*), our analyses did not support a clade uniting *dorsivittata* with the other three species. Besides, among the sampled ingroup taxa, *Mabuya dorsivittata* was the one that showed the greatest genetic distances (7.5-8.6%) from members of the *agilis/caissara/heathi* clade. This and the fact that neither the MP nor the ML analysis indicate that *M. dorsivittata* is the sister taxon to the *agilis/caissara/heathi* complex suggests that Rodrigues' (2000) tentative division of South American species into phenetic groups based mainly on superficial similarities may not reflect the actual phylogenetic relationships among these species, as cautioned by himself. Nevertheless, a more comprehensive analysis including *M. guaporicola* and other species is needed to properly assess this issue.

In both trees, topotypic *Mabuya caissara* clustered with the *M. agilis* populations from Rio de Janeiro state, with *M. caissara* genetically differing from them by only 0.0-0.5%. This suggests that *M. agilis* is paraphyletic with respect to *M. caissara*, and that both species should be considered conspecific. Rebouças-Spieker (1974) considered *M. caissara* to be more divergent from the southern São Paulo coast and Rio de Janeiro (referred to as Guanabara) forms (*M. macrorhyncha* and *M. agilis*, respectively) than the latter two are to each other. However, the data and illustrations in her work suggest a greater overall similarity between *M. caissara* and her samples from Rio de Janeiro (i.e. *agilis*). Rebouças-Spieker (1974) described a hypothetical parapatric speciation scenario, in which *M. caissara* would have diverged from an ancestor resembling the populations from southern São Paulo (*M. macrorhyncha*) and from Rio de Janeiro (*M. agilis*) during a period in which the northern São Paulo coastal area was isolated due to sea level variations. Our molecular data also indicate that *M. macrorhyncha* is not closely related to *M. agilis/caissara*, though its true affinities within the sampled ingroup taxa remain unclear. Rebouças-Spieker's (1974) hypothesis that the three forms originated from a common ancestor along the south-eastern Brazilian coast is thus not supported by our results. At that time, Rebouças-Spieker (1974) apparently believed that *M. macrorhyncha* occurred only along the southern coast of São Paulo and adjacent small islands, thus it is not surprising that she assumed that *M. macrorhyncha* must have originated in that region. *Mabuya macrorhyncha* is now known to have an extensive distribution in eastern Brazil (Rodrigues, 2000) where, except for the São Paulo populations, it is broadly sympatric with *M. agilis* and *M. heathi* (e.g. Araújo, 1994; Freire, 1996; Rocha, 2000). It is interest-

ing to note that Rebouças-Spieker (1974) pointed out that South American *Mabuya* "need to be studied comprehensively" and that "numerous detailed regional studies, backed by genetical and biochemical methods will be needed before a broad understanding comes within reach". In the present study, our genetic data did not support Rebouças-Spieker's hypothesis regarding evolution of south-eastern coastal Brazilian *Mabuya*, which further stresses the importance of detailed studies.

Mabuya heathi from the Abrolhos archipelago was found to be closely related to mainland *M. agilis* from Espírito Santo and Bahia states. Also, in both trees the Abrolhos populations clustered (though with relatively low bootstrap support, i.e. 65-73%) with the population of Prado, which is the mainland population that is geographically closest to the archipelago, among those included in our study. Genetic distances between the two Abrolhos samples (from Sueste and Siriba islands) and between the Siriba island and mainland Prado samples are very low (0.2-0.4%). This indicates that the Abrolhos populations may have originated from the mainland ones in southern Bahia, which presumably reached the archipelago quite recently, via overwater dispersal, considering that those islands are of volcanic origin and were never connected to the mainland (Martin *et al.*, 1980). Besides the fact that the Abrolhos *M. heathi* show such a low genetic variation from mainland *M. agilis* from southern Bahia, both the MP and the ML tree indicate that northern mainland *heathi* populations (Natal and Crateús) are not closely related to the Abrolhos archipelago individuals. Thus, the Abrolhos populations should be referred to as *M. agilis*, a step we have already taken in recent studies (Rocha, 2000; Rocha *et al.*, 2002b).

The two most divergent samples within the complex come from Crateús and Natal, the two northernmost and most distant localities. The Crateús and Natal populations differ genetically from each other by 1.6% and from the other *agilis/caissara/heathi* populations by 1.8-2.6% and by 1.7-2.6%, respectively. Walker & Avise (1998) used mtDNA divergence to point out that, in turtles, patterns of divergence broadly correspond to biotic entities. Specifically, they found that 90% of the putative sister species show mtDNA sequence divergence greater than 2%. However, the data of Mead *et al.* (2001) indicates that taxa that have differentiated to even greater degrees, like the two northernmost *Mabuya heathi* forms, could remain reproductively compatible and exhibit complex patterns of gene exchange. Unfortunately, we do not have sequences from populations of *M. heathi* between Natal and the presumed southern limit of the species in the mainland at Salvador, Bahia state. Analyses of such sequences could evidence if there is a tendency for genetic divergence between *heathi* and northern *agilis* populations to decrease with geographic proximity. In any case, genetic distances between *M. heathi* and *M. agilis/caissara* are relatively small, and the apparent continuous distribution of the *agilis/heathi* complex along the

Brazilian coast suggests that gene flow may occur. This phylogenetic pattern appears to correspond to "phylogeographic category III" of Avise (2000), in which 'most or all haplotypes are closely related, yet are localized geographically. The implication is that contemporary gene flow has been low enough in relation to population size to have permitted lineage sorting and random drift (or, perhaps, diversifying selection) to promote genetic divergence among populations that nonetheless were in historical contact recently'.

To sum up, our analyses support a monophyletic *Mabuya agilis/caissara/heathi* clade, within which the nominal species *agilis* and *heathi* are paraphyletic. The *agilis/caissara/heathi* clade possibly represents a single widespread species. Nevertheless, a detailed revision of those three taxa including morphological analyses and comparisons is desirable before taxonomic alterations are formally proposed. Also, further molecular analyses including sequences from more populations of *heathi* and from other *Mabuya* species could help to improve our understanding of the relationships within the *agilis/caissara/heathi* complex and between it and other congeners.

Note added in proof. While this paper was in press, an article by Whiting *et al.* (2006) focusing on the molecular phylogeny of New World *Mabuya* has appeared in print. As in our study, these authors found strong support for a clade consisting of *M. agilis* and *M. heathi* populations (*M. caissara* was not included in their analysis), with these two species not being reciprocally monophyletic. Also, their analysis supported a sister-group relationship between a *M. macrorhyncha/M. agmosticha* clade and the *M. agilis/M. heathi* cluster.

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SHORT NOTE

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**GENETIC DIVERGENCE IN THE
ENDANGERED FROG
INSUETOPHRYNUS ACARPICUS
(ANURA: LEPTODACTYLIDAE)**

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Insuetophrynus acarpicus is a poorly known frog restricted to the temperate forests of the coastal range of Chile (39° 25' S, 73° 10' W). Until recently, this species was known only from one type locality since its original description in 1970. However, in 2002 two new localities were reported, extending its distribution range to about 40 km². In order to evaluate genetic divergence, provide a preliminary evaluation of the genetic diversity of this species and the phylogenetic relationships among individuals from the three known populations, we analyzed the nucleotide variation of a fragment of the mitochondrial gene cytochrome *b*. We sampled just two or four individuals per population of this endangered frog. We found a low nucleotide divergence among populations suggesting a genetic homogeneity across the entire range. This highlights the need for further studies to define the conservation status of this endangered frog.

Key words: anuran, conservation, cytochrome *b*, population genetics

Insuetophrynus acarpicus Barrio 1970 is an endemic frog of the Chilean temperate forest, inhabiting the northern coast of Valdivia (39° 25' S, 73° 10' W), and is the only species of this monotypic genus. Studies on *I. acarpicus* have mainly focused on its natural history (Barrio, 1970; Formas *et al.*, 1980; Díaz *et al.*, 1983) and chromosomal characters (Barrio & Rinaldi, 1971; Díaz & Veloso, 1979; Díaz *et al.*, 1983). In ecological terms, this species is described as being strongly aquatic and re-

stricted to coastal streams with slopes of 19° to 38° (Díaz *et al.*, 1983). In this species larval development can take about 10 to 12 months, and recently metamorphosed individuals are found mainly in January. More details of life history and ecology of this species are provided by Díaz *et al.* (1983). Currently, *I. acarpicus* is considered to be endangered in Chile (Glade, 1993; Díaz-Páez & Ortiz, 2003), mainly due to low population abundances and its extremely narrow distribution range. In fact, for more than 30 years after its original description this species was known only from one locality, Mehuín (Barrio, 1970; Formas *et al.*, 1980). Recently, two new localities have been described for this frog (Soto *et al.*, 2002), extending its distribution range towards north-eastern Chile.

In this study, we conducted the first molecular analysis in this species, including individuals from all three localities where *I. acarpicus* has been reported. We made a preliminary evaluation of genetic variation of the cytochrome *b* gene within populations and inferred the phylogenetic relationships among the three populations. We also estimated the time elapsed since their divergence.

Specimens were obtained from the herpetological collections of the Departamento de Biología Celular y Genética of the Universidad de Chile (DBGUCH), the Instituto de Zoología of the Universidad Austral (IZUA) and the Museo de Zoología of the Universidad de Concepción (MZUC). We obtained samples of liver or toe from specimens preserved in ethanol (70%), representing the three currently known localities (Fig. 1): (1) Mehuín (39° 26' S, 73° 13' W - two specimens: DBGUCH 3133, IZUA 3249); (2) Queule (39° 24' S, 73° 13' W - four specimens: DBGUCH 3114, 3126, 3128, 3129) and (3) Colegual Alto (39° 24' S, 73° 06' W - two specimens: MZUC 26930, 26931). No other specimens were available for DNA extraction in these collections. We extracted total DNA using the phenol-chloroform (1:1) and chloroform isoamyl alcohol (24:1) method (Sambrook *et al.*, 1989). We amplified a 1050 bp fragment of the mitochondrial gene cytochrome *b* (*cyt b*) via polymerase chain reaction (PCR) using Taq DNA Polymerase (GIBCO) with primers MVZ15-L (Moritz *et al.*, 1992) and *Cytb*AR-H (Goebel *et al.*, 1999). PCRs were performed using the following thermal profile: denaturation at 94 °C (1 min 40 s), annealing at 50 °C (1 min), extension at 72 °C (2 min 30 s) for 42 cycles and 10 minutes of final extension at 72 °C. All reactions were performed in a Biometra® Personal Cycler. Double-stranded PCR products were purified with the QIAquick kit (Qiagen) and sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) in an ABI Prism 3100 automated sequencer. Sequences were aligned using BioEdit software (Hall, 1999) with the ClustalW option and compared by eye. We also checked the sequences using both DnaSP 3.53 (Rozas & Rozas, 1999) and BioEdit programs to translate them into amino acids. All sequences were submitted to

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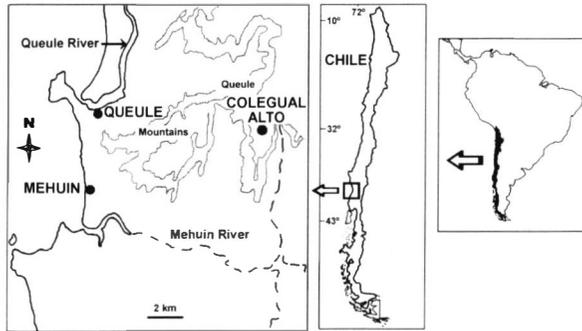


FIG. 1. Map showing the location of the three known localities of *Insuetophrynus acarpicus*. Dotted lines correspond to major rivers and continuous lines represent altitudinal contours.

GenBank (accession numbers AY386396-AY386401 and AY691185-AY691187).

We applied parsimony and maximum likelihood analyses; in both cases we used exhaustive search option. Support of the nodes was assessed by non-parametric bootstrap with 1000 pseudoreplicates. Phylogenetic analyses were performed using PAUP* 4b10 software (Swofford, 2001). In all analyses we used as outgroup one individual of *Eupsophus* sp. (DBGUCH 3273) from the Region VII, Chile. *Eupsophus* and *Insuetophrynus* are thought to be basal members of Leptodactylidae and related genera according to previous systematic studies (Lynch, 1978; Díaz, 1986). To calibrate a molecular clock for the sequences of *I. acarpicus* we evaluated the sequence neutrality using a hierarchical likelihood ratio test (hLRT) (Huelsenbeck & Crandall, 1997). Since there are no molecular clock calibrations available for Leptodactylidae, and the evolutionary relationships with other frog families remain unclear, we applied a standard nucleotide substitution rate of 0.5-1% per lineage per million years. This calibration value represents an estimate described for vertebrate mitochondrial genes (Moritz *et al.*, 1987). We obtained an alignment of 707 bp with 170 variable sites among *Insuetophrynus* and *Eupsophus* sequences, and 14 variable sites among *Insuetophrynus* sequences, all of them parsimony informative sites. Only three haplotypes were observed for *I. acarpicus*, with no intrapopulation variation. Proportion of variable sites (p-distances) of haplotypes and geographic distances among *I. acarpicus* localities are shown in Table 1. Phylogenetic analyses recovered one tree for both reconstruction methods parsimony and maximum likelihood. Hence, we show the maximum likelihood tree (Fig. 2; $-\ln L = 1555.3914$) constructed with the GTR+G substitution model which was obtained using Modeltest 3.06 and AIC criterion (Posada & Crandall, 1998).

Phylogenetic analyses separated *I. acarpicus* individuals in two groups, one including individuals from Queule and the other including those from Mehuín and Colegual Alto. At the population level, the Queule locality showed the same level of divergence (1.84%; Table 1) when was compared with localities of Mehuín and

TABLE 1. Proportion of variable sites (p-distances) for *Insuetophrynus acarpicus* cytochrome *b* sequences (below diagonal) and geographic distances (km) among localities (above).

	1	2	3
1. Queule		4	11.5
2. Mehuín	0.0184		10.7
3. Colegual Alto	0.0184	0.0028	

Colegual Alto. In contrast, between Colegual Alto and Mehuín populations we found a smaller amount of differentiation (0.28%; Table 1). These levels of divergence are similar than those reported in other amphibian species. For cytochrome *b*, Tan & Wake (1995) reported for *Taricha torosa* a population divergence between 0.6- 2.5 %. Instead, Shaffer *et al.* (2004) reported intraspecific divergence rates between 0-5 % for five species of genus *Rana*.

The hLRT did not show a significant difference ($P > 0.05$) between topologies with enforced vs. non-enforced molecular clock, therefore we cannot reject the neutral evolution of sequences. Using divergence rates of 0.5 and 1% per lineage per million years, we postulated that divergence times between Queule and Mehuín-Colegual Alto populations occurred between 1840000 and 920000 years ago, respectively. Instead, divergence between Mehuín and Colegual Alto populations could have occurred around 280000 and 140000 years ago. These divergence times suggest that separations among these populations occurred in the Pleistocene. Although there is a considerable literature about paleoclimatic reconstructions of the south of Chile, most of them are related to Holocene period (Clapperton, 1994; Veit, 1994; Villagrán, 1994, 2001; Potts & Behrensmeyer, 1992; among others) and there is no paleoclimatological evidence from this region about Pliocene-middle Pleistocene (Hinojosa & Villagrán, 1997). This fact does not allow us to discuss

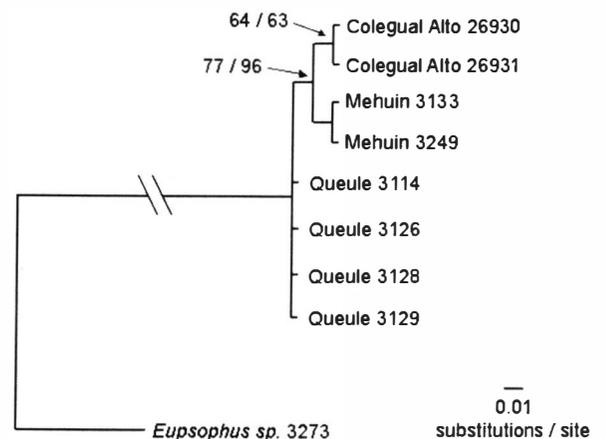


FIG. 2. Maximum likelihood (ML) tree for cytochrome *b* sequences of eight *Insuetophrynus acarpicus* individuals from the three known localities. Bootstrap values are indicated above each node of the tree, maximum likelihood (ML) and maximum parsimony (MP), respectively (ML/MP). Only values over 50% are shown.

in detail the observed population divergence in an historical context. In this respect, we only can say that during glacial events, processes of expansion and contraction of habitat could have influenced the differentiation of *I. acarpicus*. Our phylogenetic analyses show that Mehuín and Colegual Alto diverged after than Queule population, however, because our knowledge of distribution of *I. acarpicus* is fragmentary, we cannot say that Queule population gave rise to others two. In this sense we propose that Queule population belong to an ancestral lineage in comparison to other two populations. On the other hand, the existence of a common drainage area for Colegual Alto and Mehuín populations could explain why these populations appear separated from Queule population which belongs to another watershed. Accordingly, it is possible that Colegual Alto and Mehuín were connected by streams and gorges in this region and, because this species is strongly aquatic and restricted in its distribution to streams (Díaz *et al.*, 1983), the mechanism of colonization could be related to passive dispersion via stream running. Finally, given that this genus inhabits a narrow geographic range (about 40 km²; Fig. 1) without naturally protected areas, and where the human population is currently in expansion and strongly modifying the environment, it is important to continue studying this species, focusing on the development of a more extensive survey programme to find new localities inhabited by this frog. In fact, the historic absence of records for this species is a strong indicator of low population abundances, especially since other rare frog species (e.g. *Eupsophus migueli*) have been collected in the same areas (Méndez *et al.*, *in press*). This information will be highly relevant for understanding the genetic diversity and conservation status of *I. acarpicus*.

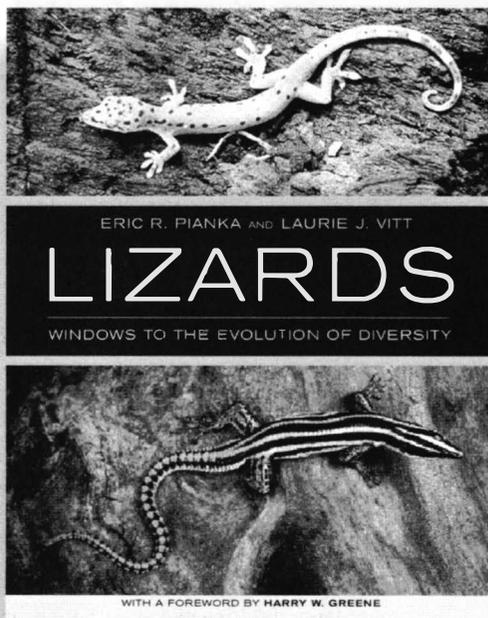
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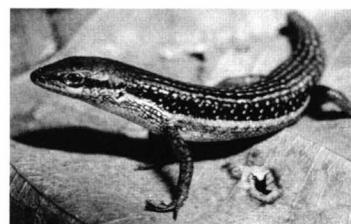
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THE HERPETOLOGICAL JOURNAL

Volume 16, Number 1 2006

CONTENTS

Full papers

- | | | |
|---|---|-------|
| A new species of <i>Mabuya</i> (Reptilia, Squamata, Scincidae) from the Caribbean Island of San Andrés, with a new interpretation of nuchal scales: A character of taxonomic importance | A. MIRALLES | 1-7 |
| Sexual dimorphism in two species of European plethodontid salamanders, genus <i>Speleomantes</i> | S. SALVIDIO &
R. C. BRUCE | 9-14 |
| Seasonal and hourly patterns of reproductive activity in <i>Scinax trapicheiroi</i> (Anura, Hylidae), Rio de Janeiro State, South-eastern Brazil | M. VAN SLUYS,
M. RICO &
C. F. D. ROCHA | 15-20 |
| Chorus organization of the leaf-frog <i>Phyllomedusa rohdei</i> (Anura, Hylidae) | H. WOGEL,
P. A. ABRUNHOSA &
J. P. POMBAL JR. | 21-27 |
| Changes in community composition, habitats and abundance of snakes over 10+ years in a protected area in Italy: conservation implications | E. FILIPPI &
L. LUISELLI | 29-36 |
| Ecology of the colubrid snake <i>Pseudablabes agassizii</i> in south-eastern South America | O. A. V. MARQUES,
R. J. SAWAYA,
F. STENDER-OLIVEIRA &
F. G. R. FRANCA | 37-45 |
| Interpopulation differences in water-seeking behaviour in the green toad <i>Bufo viridis</i> | J. HOFFMAN | 47-53 |
| Pivotal temperature for green sea turtles, <i>Chelonia mydas</i> , nesting in Suriname | M. H. GODFREY &
N. MROSOVSKY | 55-61 |
| Influence of small-scale fires on the populations of three lizard species in Rome | L. RUGIERO &
L. LUISELLI | 63-68 |
| Review of the reintroduction programme of the mugger crocodile <i>Crocodylus palustris</i> in Neyyar reservoir, India | E. A. JAYSON,
C. SIVAPERUMAN &
P. PADMANABHAN | 69-76 |
| Presence and absence of the cement gland in foam-nesting leptodactylids (Anura: Leptodactylidae): implications for the transition to terrestrial development | J. R. DOWNIE &
M. NOKHBATOLFOGHAI | 77-81 |
| Molecular phylogeny of Brazilian <i>Mabuya</i> (Reptilia, Squamata, Scincidae) of the <i>agilis/caissara/heathi</i> complex | D. VRCIBRADIC,
P. MAUSFELD-LAFDHIYA &
C. F. D. ROCHA | 83-91 |
| <h3>Short Note</h3> | | |
| Genetic divergence in the endangered frog <i>Insuetophrynus acarpicus</i> (Anura: Leptodactylidae) | M. A. MÉNDEZ,
F. TORRES-PÉREZ,
C. CORREA,
E. R. SOTO,
J. J. NUÑEZ,
A. VELOSO &
J. ARMESTO | 93-96 |