

PHYLOGENETIC RELATIONSHIPS OF LIZARDS OF THE  
*LIOLAEMUS PETROPHILUS* GROUP (SQUAMATA, LIOLAEMIDAE),  
WITH DESCRIPTION OF TWO NEW SPECIES  
FROM WESTERN ARGENTINA

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**ABSTRACT:** We describe two new species of lizards of the genus *Liolaemus* from western Argentina. Both species belong to the *petrophilus* group and are easily distinguished from other members by a combination of chromatic and squamation characters. We used sequences of the mitochondrial *cyt-b*, 12S, and ND4 and the nuclear *C-mos* genes to infer the phylogeny of described species of the group. We found evidence for a monophyletic *petrophilus* group within the *L. elongatus-kriegi* complex. The *petrophilus* group includes *Liolaemus petrophilus* and two strongly supported clades, one containing the species distributed in the north, which includes one of the new species, *L. talampaya*; the second clade includes the species distributed in the south, including the new species, *L. gununakuna*.

**Key words:** Argentina; Liolaemidae; *Liolaemus gununakuna* sp. nov.; *Liolaemus talampaya* sp. nov.; New species; Squamata

IN A RECENT mitochondrial DNA phylogenetic analysis of several closely related *Liolaemus* species, evidence was presented to support a *Liolaemus elongatus-kriegi* complex (Morando et al., 2003) which was morphologically defined by Avila et al. (2003). Within this complex, three main groups were identified: the *elongatus* group as the sister clade of the *kriegi* group, and the *petrophilus* group. There have been several previous hypotheses about relationships among species now included in this complex. The species now included in the *petrophilus* and *elongatus* groups have been included together in a larger *elongatus* group (Espinoza and Lobo, 2003; Espinoza et al., 2000; Lobo, 2001; Schulte et al., 2000); but we found evidence (Morando et al., 2003; this work) for a separate *petrophilus* group and a more restricted definition of the *elongatus* group, closely related to the *kriegi* group (not included in the previous wider definition of the *elongatus* group). This close relationship between the *elongatus* and the *kriegi* groups was previously proposed by Cei (1979).

The *petrophilus* group is comprised of species almost exclusively confined to Sub-

andean or Patagonian Steppe environments along the eastern slope of the central Argentinian Andes and related rangelands farther to the northwest and extends to the volcanic tablelands of northern Patagonia in the south (Fig. 1). These lizards are medium-sized (65–112 mm snout–vent length), long-tailed, viviparous, insectivorous, and almost exclusively saxicolous; they are found in rocky environments between 350–4000 m. Eight species are recognized in this group (including the species described herein), but at least four other populations are identified as candidate species in need of further study. Extensive field work carried out in the last 7 yr in Patagonia and western Argentina allowed us to obtain samples of several undescribed species, two of which we describe here; these are presented in the context of a phylogenetic analysis of the *petrophilus* group using mitochondrial and nuclear markers.

#### MATERIALS AND METHODS

We examined sample series of the species determined by Morando et al. (2003) as members of the *petrophilus* group (Table 1, Appendix I) from the herpetological collections of Fundación Miguel Lillo (FML), Argentina; Monte L. Bean Museum, Brigham

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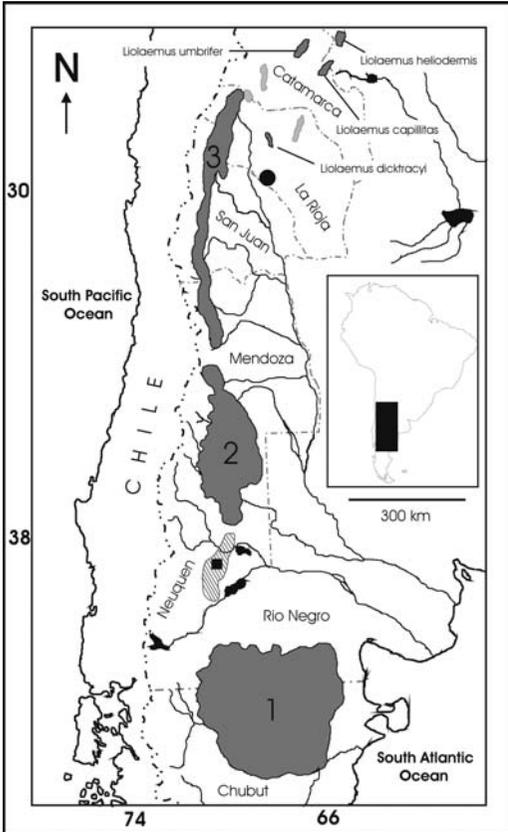


FIG. 1.—Distribution of the *petrophilus* group in Argentina: black square, type locality of *Liolaemus gumunakuna*, dashed area, known distribution of this species. Black dot: type locality of *L. talampaya*. Dark gray areas, known distribution for *L. petrophilus* (1), *L. austromendocinus* (2), and *L. cf. elongatus* (3). Light gray areas, known distributions for the species *L. capillitas*, *L. dicktracyi*, *L. heliodermis*, and *L. umbrifer*; lightest areas without reference marks identify populations that may represent undescribed species of the *petrophilus* group.

Young University (BYU); Museo de La Plata, Universidad Nacional de La Plata (MLP.S); Museum of Vertebrate Zoology, UC–Berkeley (MVZ); and the field collection of L. J. Avila and M. Morando (LJAMM), now housed in the Centro Regional de Investigaciones Científicas y Transferencia Tecnológica La Rioja (CRILAR–CONICET).

#### Morphological Analyses

Morphological characters follow Smith (1946) and recent treatments of the genus

*Liolaemus* by Etheridge (1993, 2001) and Espinoza et al. (2000); neck-fold terminology follows Frost (1992). Scale characters were observed with a Leica Zoom 2000 (10–40×) binocular stereomicroscope. Measurements were taken with a Mitutoyo® dial caliper to the nearest 0.1 mm. Descriptions of color in life are based on notes taken in the field and color photographs of recently captured animals. Additional specimens were examined after fixation in 10–20% formalin and preservation in 70% ethanol. Data for *Liolaemus heliodermis* were obtained from Espinoza et al. (2000). Chromosome preparations were made from bone marrow, spleen, and testis using a cell suspension technique (Kasahara et al., 1983) and stained with Giemsa.

#### Molecular Procedures

Protocols for DNA extraction, mtDNA primer descriptions, PCR, and sequencing procedures followed Morando et al. (2003) for the cytochrome-*b* (811 bp), ND4 (833 bp), and 12S (850 bp) regions. For the nuclear gene *C-mos*, we used primers G73 and G78 from Saint et al. (1998) under PCR conditions: initial denaturation at 93 C for 3 min; 40 cycles of denaturation at 94 C for 1 min, annealing at 52 C for 1 min, and elongation at 72 C for 1 min; and final elongation at 75 C for 5 min. This 509 bp PCR product was sequenced in the same way as the mtDNA genes. Sequences were deposited in GenBank under accession numbers AY367790 to AY367904. See Appendix II for specimens used for molecular analyses.

#### Alignment

Sequences were edited and aligned using the program Sequencher 3.1.1 (Gene Codes Corporation Inc., 1995), and the protein coding regions *cyt-b* and ND4 were translated into amino acids for confirmation of alignment. Divergence was low for the 12S fragment. The number of indels was small (11) and, in most cases (8), only a single base in length, and in three cases two base pairs in length. Alignment of this region was performed with CLUSTAL X (Thompson et al., 1997), using the default settings for gap and mismatch penalties, with subsequent manual adjustments to minimize the number of independent indels (all of which were coded as fifth character states). Missing

TABLE 1.—Diagnostic characters for currently recognized members of the *Liolaemus petrophilus* group. Data for *L. heliodermis*, follow Espinoza et al. (2000). Description of body color and patterns was taken from live lizards.

Character	<i>austromendocinus</i> (n = 40)	<i>capillitas</i> (n = 11)	<i>dicktracyi</i> (n = 13)	<i>heliodermis</i> (n = 3)	<i>gununakuna</i> (n = 19)	<i>talamayaya</i> (n = 7)	<i>umbrifer</i> (n = 11)	<i>petrophilus</i> (n = 69)
Mid-body scales	63–81	60–70	61–73	62–69	84–97	58–69	57–72	75–88
Keels on dorsal scales	Weak	Distinct	Weak/ distinct	Weak	Distinct	Distinct	Weak	Distinct
Preloacal pores	2–4	0–4	3–4	3–4	1–3	3–5	3–4	2–5
Head color	Brownish gray	Brown/ black	Black	Black	Iridescent yellow	Brown dark/brown	Brown/ black	Ochre/yellow
Body color	Brownish gray	Brown to black	Indigo/light blue	Sulfur yellow	Iridescent yellow	Tan/light brown	Brown/ black	Ochre/yellow, dark brown and yellow
Dorsal body pattern	Indistinct	Indistinct	Indistinct	Indistinct	Transverse bars	Indistinct	Indistinct	Indistinct to transverse bars
Tail rings	Weak to distinct	Absent	Absent	Absent	Distinct	Weak to distinct	Absent	Distinct

data were present in a few sequences, and these were coded as “?”.

#### Phylogenetic Analyses

Separate Bayesian analyses (based on GTR + I +  $\Gamma$  model of evolution) (Gu et al., 1995; Yang, 1994) were determined using ModelTest (Posada and Crandall, 1998) and were performed for each gene partition using MrBayes 2.0 (Huelsenbeck and Ronquist, 2001) to detect potential areas of incongruence (Wiens, 1998). The specific a priori parameter values were uniform and were estimated as part of the analysis. In order to more thoroughly explore the parameter space, we ran Metropolis–Coupled Markov Chain Monte Carlo simulations (MCMCMC) with four incrementally heated chains, using the default values. From a random starting tree, we ran  $1.0 \times 10^6$  generations and sampled the Markov chains at intervals of 100 generations to obtain 10,000 data points. We determined when stationarity was reached (in order to discard the “burn-in” samples) by plotting the log-likelihood scores of sample points against generation time; when the values reached a stable equilibrium, stationarity was assumed. The equilibrium samples (the trees retained after burn-in) were used to generate a 50% majority rule consensus tree. The percentage of samples that recover any particular clade on this tree represents the posterior probability

(PP) of the clade. The PP values are the  $P$ -values, and we consider  $P \geq 95\%$  as evidence of significant support for a clade (Huelsenbeck and Ronquist, 2001). To avoid a local entrapment, we ran two independent analyses and compared these for convergence to similar mean log-likelihood values (Huelsenbeck and Bollback, 2001; Leaché and Reeder, 2002).

The combined data set of 3003 bp was used for phylogenetic analyses. We used PAUP\* (version 4.0b4b; Swofford, 2001) under maximum parsimony (MP) and maximum likelihood (ML) criteria, MrBayes 2.0 (Huelsenbeck and Ronquist, 2001) for the Bayesian approach, and MetaPiga v1.0.2b (available at: [www.ulb.ac.be/sciences/ueg](http://www.ulb.ac.be/sciences/ueg)) to implement the genetic algorithm recently described by Lemmon and Milinkovitch (2002).

For MP analysis, all characters were equally weighted, and we conducted a heuristic search with 10,000 replicates of random addition with TBR branch-swapping and gaps coded as missing data. Bootstrap values (Felsenstein, 1985) were calculated using 10,000 pseudo-replicates. For ML analysis, the combined data set was analyzed under the GTR + I +  $\Gamma$  (Gu et al., 1995; Yang, 1994) and selected using Model Test (Posada and Crandall, 1998) that employed a heuristic search with 10 replicates and TBR branch-swapping. Because of computational limitations imposed by ML estimation, we used PAUP\* to perform four separate



FIG. 2.—Upper: *Liolaemus gununakuna*, adult male (FML 12717), from 2 km SE La Amarga, Zapala Department, Neuquén Province, Argentina; lower: type locality of *Liolaemus gununakuna*, landscape in the type locality 2 km SE La Amarga, Zapala Department, Neuquén Province, Argentina.

searches with 50 replicates each, in a non-parametric bootstrap analysis (Felsenstein, 1985), and then combined the total 200 pseudoreplicates to obtain the bootstrap proportions. Settings for all these four searches were the same: maxtrees = 1000, timesearch limit = 1000, and the same model of evolution used for the ML tree search. All ML analyses were performed on a IBM Sp2 supercomputer at Brigham Young University.

For Bayesian analysis, we again used the GTR + I +  $\Gamma$  model (Gu et al., 1995; Yang, 1994), with two independent analyses of  $1.5 \times 10^6$  generations. A majority rule consensus tree was generated from the equilibrium samples.

We used MetaPiga to implement a new genetic algorithm (the metapopulation genetic algorithm); this is a heuristic approach that improves the speed with which maximum likelihood (ML) trees are found. It also provides an estimate of the posterior probability

distribution of possible trees, with clade frequencies closely approximating their posterior probabilities (Lemmon and Milinkovitch, 2002). To obtain a consensus tree based on this algorithm, we ran MetaPiga for 10 times with 10 populations each and generated a consensus tree from the 100 recovered trees (as suggested by Lemmon and Milinkovitch, 2002).

#### SPECIES DESCRIPTION

##### *Liolaemus gununakuna* sp. nov.

*Holotype*.—Fundación Miguel Lillo (FML) 12717 (Fig. 2), an adult male from 2 km SE La Amarga (39° 06' S, 69° 34' W), Zapala Department, Neuquén Province, Argentina; collected by L. J. Avila and M. Morando; 7 January 2000.

*Paratypes*.—BYU 47309–11, FML 12719, LJAMM 287 (females), LJAMM 2690 (male) from Los Candeleros, SE Cerro Lotena, 45 km SW Cutral C6; collected by L. J. Avila, 29 November 1995; FML 12718, LJAMM 2438 (female) rocky hills near La Amarga, collected by L. J. Avila, M. Morando, and D. R. Perez, 2 May 1998; FML 12720 (male), MLP.S 2352–53 (females), same data as holotype. Specimens FML 13043–44, LJAMM 143, 265, 279, 2440 (females), LJAMM 266 (male), Bosque Petrificado, collected by L. J. Avila, M. Morando, and D. R. Perez, 1 May 1998. All localities are in Zapala Department, Neuquén Province, Argentina.

*Diagnosis*.—*Liolaemus gununakuna* is a member of the *petrophilus* group (Table 1), which is itself nested within the *Liolaemus elongatus-kriegi* complex (Morando et al., 2003), and differs from all other members of the group in its distinctive yellow-green, iridescent background color pattern (Fig. 2). *Liolaemus petrophilus* has slightly larger dorsal scales and a lower midbody scale count than *L. gununakuna*, and, in background coloration, *L. petrophilus* varies from dark brown to ochre/yellow/green on the body with a brownish gray head, but it never has iridescent green coloration; transverse body bars in *L. petrophilus* vary from indistinct to very evident, while black body bars are always present and well defined in *L. gununakuna*. *Liolaemus austromendocinus* has weakly

keeled dorsal body scales, lower and non-overlapping dorsal scale counts, pale brown dorsal coloration with a indistinct pattern that is covered by small scattered irregular black flecks, and the tail is ringed by indistinct brown stripes. *Liolaemus capillitas*, *L. dicktracyi*, *L. talampaya* sp. nov., and *L. umbrifer* all have lower and nonoverlapping midbody scale counts and never have color patterns of transverse black bars or well defined ringed tails; all of these species also have a darker background body color than *L. gununakuna*. *Liolaemus heliodermis* has sulfur–yellow torso and black head in males, but this pattern of sexual dichromatism is not present in *L. gununakuna*.

*Description of holotype*.—Adult male (Fig. 2); 92.9 mm snout–vent length (SVL); tail length 140.0 mm, complete, non-regenerated. Axilla–groin distance 47.4 mm. Head length 21.3 mm (from anterior border of tympanum to tip of snout), 18.0 mm wide (at anterior border of tympanum), 11.6 mm high (at anterior border of tympanum). Snout length 7.3 mm (posterior margin of canthal to tip of snout). Interorbital distance 1.8 mm. Eye–nostril distance 8.0 mm. Orbit–auditory meatus distance 7.8 mm. Orbit–tip of snout distance 8.4 mm. Forelimb length 35.0 mm. Hindlimb length 61.8 mm. Tibial length 20.0 mm. Foot length 28.7 mm (ankle to tip of claw on fourth toe).

Dorsal head scales smooth, 16 between occiput at level of anterior border of tympanum to rostral, pitted with numerous scale organs in snout region. Rostral scale wider (3.6 mm) than high (1.6 mm). Two postrostrals, together with anterior lorilabial, separate nasal scales from rostral. Nasal scales longer than wide, irregularly elliptic; nostril over one-half length of nasal, posterior in position. Scales surrounding nasals 6–7 (right/left). Five internasals, right subdivided in two small scales. Frontonasal 8, irregular; prefrontals 5, with scar damage. Five frontal scales, two azygous frontal scales, irregular, followed by 2 scales and a small scale on the right. Interparietal subpentagonal, surrounded by seven scales; five smaller in front and sides, two larger in back. Parietals smooth, flat, irregular shaped. Supraorbital semicircles 12 scales on each side. Circumorbitals: 12–13. Transversally expanded supraoculars 6–7 (right/left). Smaller

lateral supraoculars: 18–13 (right/left). Anterior canthal wider than long, separate from nasal by two postnasals. Posterior canthal longer than wide, overlapping anterior superciliary. Superciliaries 6–7 (right/left), flattened and elongated, anterior four broadly overlapping dorsally. Two loreal scales, flat (small accessory scale in right side). Orbit with 17 upper and 15 lower ciliaries. Orbit diameter  $3.1 \times 4.6$  mm. Preocular small, unfragmented, longer than wide. Subocular scale elongated, six times longer than wide ( $6 \times 1$  mm). Postocular slightly overlapping subocular, small, almost one-fourth length of subocular. A longitudinal ridge along upper margin of three ocular scales. Palpebral scales small, irregular, flat. Lorilabials flat, 10 on each side, 5–4 in contact with subocular, similar or slightly higher than supralabials. Supralabials five, first four flat, last one convex, followed by one small postlabial scale. Fifth supralabial curved upward posteriorly but not in contact with subocular. Temporals smooth, flat to concave, subimbricate. Upper temporals with slightly swollen, some with slightly blunt keel. Anterior auriculars convex, smaller than adjacent posterior temporals. Posterior auriculars small, granular. Three scales along anterior border of tympanum project outward, one larger, white. External auditory meatus higher (3.7 mm) than wide (2.2 mm). Lateral scales of neck granular with slightly inflated skin. Antehumeral, gular, longitudinal, and postauricular distinct, oblique less conspicuous, rictal not present. Forty-six scales between tympanum and antehumeral fold (counted along longitudinal fold). Scales of dorsal neck region similar to body dorsals. Eighty-eight dorsal scales between occiput and anterior surface of thighs.

Mental scale wider (3.9 mm) than high (2.3 mm), in contact with four scales. Mental followed posteriorly by two rows of five chinshields. Six infralabials on each side, two times wider than supralabials. Gular scales smooth, flat, imbricate, with rounded posterior margins. Scales of throat between chinshields slightly juxtaposed, becoming slightly imbricate toward auditory meatus. Fifty-seven gulars between tympana. Infralabials separated from chinshields by one to three rows of smaller scales.

Dorsal body scales rhomboidal, some lanceolate, imbricate, with a distinct keel. At

midbody, dorsal scales grade laterally into slightly smaller, smooth scales. Scales immediately anterior to, above, and posterior to forelimb and hind limb insertion small, smooth, almost granular, and nonoverlapping. Ventral body scales smooth, flat, imbricate, same size or a little larger than dorsal scales. Ninety scales around midbody; mid-dorsal scales between occiput and anterior margins of hind limb articulations 89; scales between mental and preloacal pores 128. Scales of cloacal region about equal or smaller in size to ventral body scales. Two evident preloacal pores.

Anterior suprabrachials rhomboidal to lanceolate, faintly keeled, about 1.5 times as large as dorsal body scales, grading into rounded and smooth scales posteriorly. Posterior suprabrachials smaller, smooth, becoming granular near axilla. Anterior antebrachials similar to suprabrachial, but some scales with a notch in the tip. Posterior antebrachials smaller, smooth, rounded. Supracarpals rounded to rhomboidal, smooth. Infracarpals strongly imbricate, rhomboidal, keeled, some with short mucron. Pre- and postdigital scales of manus smooth. Subdigital lamellae with three blunt keels, each terminating in a short mucron, numbering: I: 17, II: 28, III: 23, IV: 18, V: 12. Claws robust, moderately curved, opaque brown, similar in length to penultimate phalanx.

Anterior suprafemorals twice as large as dorsal body scales, rhomboidal to lanceolate, keeled or smooth, some with a small notch in tip, grading posteriorly into smaller, smooth, and rounded scales. Posterior suprafemorals small, granular shape. Supratibial rhomboidal to lanceolate, keeled, grading into rounded, smooth, posterior supratibials, same size as ventral body scales. Supratarsal and first supradigital keeled, middle and distal supradigital smooth. Infratarsal small, rhomboidal, imbricate, keeled, some with a small mucron. Subdigital scales keeled, most with two or three keels, not mucronate, numbering: I: 14, II: 20, III: 25, IV: 33, V: 18. Claws robust, moderately curved, opaque brown. Dorsal and lateral caudals keeled, ventral smooth.

*Color in life.*—Ground color of head, body, limbs, and tail (except some ventral areas) iridescent yellow-green (Fig. 2). Dorsal head scales with black margins, scarce in the

frontonasal-prefrontal area and becoming more extended between frontal to occiput, laterally reaching circumorbital scales. Rostral, postrostral, nasal, loreal, canthal, supercilia-ries, suboculars, lorilabials, and supralabials scales completely iridescent yellow-green. Black lines or irregularly distributed black scales in temporal area. From occiput to caudal annuli, a series of transversal, irregular, dark bars, 3 to 1 scale wide, partially fused along vertebral line ("tigroid pattern"). Each bar extended laterally to reach ventral scales, but with irregular fusions or splitting up in the dorsolateral and lateral fields. Bars from occiput to first caudal annulus: 23-24. Black bars completely fused in the area surrounding the thigh's insertions, forming a black net with small yellow spots. Thighs with an irregularly ringed pattern of black bars in dorsal areas. Tail with a series of 35 black rings, 1-2 scale wide, ventrally incomplete or very subtle in the proximal third, complete and evident to tip. Chest and gulars scales pale yellow-green. Belly yellow-white medially, grading into yellow on sides. Lower portion of belly, ventral femoral areas, and preloacal regions with deep yellow or yellow-white scales, not iridescent, very distinct from iridescent yellow-green general ground color.

*Color in preservative.*—All yellow-green iridescence is lost and yellow-green coloration fades to green-blue. Black patterns maintained, and throat, jaw, thigh, and some caudal scales darken to light gray. Belly scales turn black in an irregular pattern. Yellow-white scales of the lower belly, femoral, and preloacals regions turn creamy white.

*Variation in paratypes.*—In three males; SVL (mean  $\pm$  SD (range)): 91.0  $\pm$  3.38 (87.4-94.1). Axilla-groin distance: 34.4  $\pm$  7.1 (26.4-39.9). Head length: 20.6  $\pm$  1.15 (19.5-21.8). Head width: 17.0  $\pm$  1.27 (15.7-18.3). Forelimb length: 56.9  $\pm$  3.0 (53.6-59.6). Hind limb length: 35.3  $\pm$  1.8 (33.8-37.4). Midbody scales: 85-90. Dorsal scales: 83-90. Ventral scales: 108-112. Preloacal pores: 1-3. In fifteen females; SVL (mean  $\pm$  SD (range)): 85.0  $\pm$  8.0 (70.3-97.5). Axilla-groin distance: 38.8  $\pm$  2.0 (36.1-41.4). Head length: 17.2  $\pm$  4.7 (17.5-19.2). Head width: 15.1  $\pm$  1.2 (14.3-17.1). Forelimb length: 31.9  $\pm$  1.8 (28.3-33.7). Hind limb length: 52.1  $\pm$  3.2 (46.8-55.2). Midbody scales: 84-94. Dorsal scales: 85-103.

Ventral scales: 109–122. Preloacal pores not present in females.

Interparietal scale usually irregularly shaped, bordered by 5–9 scales. Supraoculars scales: 6–5. Number of scales around nasal 5–6. Nasal only in slight contact with rostral in one individual. Scales between rostral and frontal: 5–7. Supralabial scales 4–6. Infralabial scales 5–6. Lorilabials: 7–11. Lorilabials in contact with subocular: 3–4. Fourth finger lamellae counts: 19–22. Fourth toe lamellae counts: 25–32. Gular scales between both auditory meatus: 58–64. Internasal scales 4–6. Upper temporals smooth to slightly keeled, lower temporals smooth. Parietals smooth, slightly convex.

In some lizards, prefrontal, frontal, interparietal, and parietal scales are completely black, without yellow areas, and, in some others, a wide black dorsal vertebral stripe (9–12 scale wide) is very evident when all scales between the neck and tail are completely or partially black. Occasionally this black stripe merges with black head scales, becoming a continuous longitudinal black stripe between the head and first third portion of the tail. Shape and pattern of division/fusion of body black bars and caudal rings vary between individuals; melanistic extension on the head, neck, limbs, and tail varies in intensity: in some lizards, black head scales are concentrated on the top, leaving the majority of the head completely yellow–green. In these cases, scale organs become very evident. Extension of the yellow–white color in the preloacal and femoral areas varies among individuals, reaching the midbody in some but, in others, being restricted to the femoral and preloacal areas.

*Sexual dimorphism.*—As in other members of this complex, no body–size dimorphism or squamation differences were observed. The base of tail of males is expanded laterally, and the yellow–orange preloacal pores are larger. Preloacal pores not present in females.

*Karyology.*—Twenty selected somatic metaphase and meiotic plates were analyzed and photographed from three lizards. The diploid complement of *Liolaemus gununakuna* has  $2N = 32$  chromosomes, with six pairs of metacentric or submetacentric macrochromosomes and 20 microchromosomes. The second macrochromosome pair has a secondary constriction at the tip of the long arm; the

karyotype is similar to other members of the *Liolaemus elongatus–kriegi* complex (Morando et al., unpublished data). In meiotic cells, 6 macrobivalents and 10 microbivalents were observed; additional details will be provided in some of our future publications.

*Etymology.*—Named to honor the members of the northern populations of the Tehuelches aboriginal people (Güntin–a–küna) who inhabited northern Patagonia until the arrival of Araucanian tribes from Chile and, later, western civilization; this culture is now almost extinct.

*Distribution.*—*Liolaemus gununakuna* is known from several localities in Neuquén Province, in the Picún Leufú and Zapala Departments (Fig. 1). It may be expected to occur south of this area in the Department Catan Lil and north in the Department Añelo, where habitat similar to the type locality exists (Fig. 2). Sightings and photographic records were communicated to L. J. Avila by oil workers and biologists who referred to this lizard as the “green lizard”. The distribution area is known physiographically as “Patagonia extraandina” and is composed of two different geological regions: a plateau zone and a lowland zone. *Liolaemus gununakuna* appears to be restricted to the lowland zone, which is characterized by small hills of highly folded Jurassic or Cretaceous sediments that are modified by extensive wind and water erosion and contain surface boulders surrounded by sandy soils. The plant community is ecotonal between the Austral Monte and Patagonian Steppe, with dominant vegetation composed by *Atriplex lampa*, *Berberis comberi*, *Chuquiraga hystrix*, *Colliguaya intergerrima*, *Condalia microphylla*, *Haploppapus pectinatus*, *Larrea divaricata*, *Prosopis flexuosa*, *P. denudans*, *Retanilla patagonica*, and some grasses such as *Stipa* sp. (Roig, 1998).

*Natural history.*—*Liolaemus gununakuna* was first collected in a desert landscape known as Los Candeleros, south of Cerro Lotena, Picun Leufu Department. In this area, *L. gununakuna* is very rare and is only found in isolated red rocky outcrops with big boulders and crevices. These rocky outcrops extend in a north–to–south line for several kilometers and form isolated islands surrounded by a flat terrain with soft sandy soil; the majority of lizards were observed on these boulders. Only

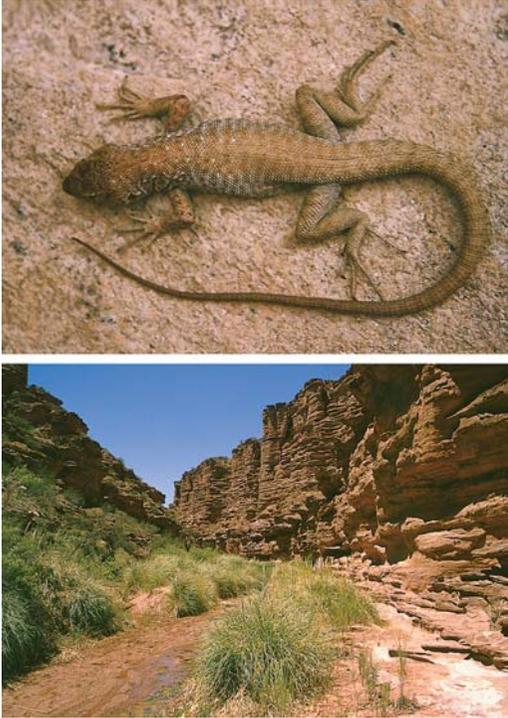


FIG. 3.—Upper: *Liolaemus talampaya*, adult male (FML 13411), from Rio Las Yeguas, Sierra de los Tarjados, Parque Nacional Talampaya, Felipe Varela Department, La Rioja Province, Argentina; lower: type locality of *Liolaemus talampaya*, canyon of Rio Las Yeguas, Sierra de los Tarjados, Parque Nacional Talampaya, Felipe Varela Department, La Rioja province, Argentina.

a few lizards were observed running or foraging between small bushes in sandy areas in close proximity to the outcrops, but they usually retreated to the protection of the rocks if an attempt was made at capture. *Liolaemus darwinii* and an undescribed species of the *boulengeri* group were captured in the same place, and *L. gracilis* and *Homonota darwinii* were observed in bordering bushes and below rocks. At the type locality, near the town of La Amarga, we found only *Liolaemus donosobarrosi* in sympatry with *L. gununakuna*. The landscape is completely different, with small, flat, rocky hills with dispersed fossil tree trunks and small ridges of sedimentary rock.

Little more than anecdotal comments can be offered regarding the biology of the species. In the Los Candeleros area, lizards were observed being active only between 1030 and 1300 h in 3 d of active search (28–30 November 1995); at the type locality, lizards

were observed with a bimodal pattern of activity between 1000–1300 h in the morning and 1600 to 1800 h in the afternoon (1–2 May 1998; 22 March 2000). When active, lizards moved across a rocky substrate and basked on horizontal surfaces on rocks. Upon disturbance, *Liolaemus gununakuna* usually retreated into crevices or below flat stones. As with other saxicolous *Liolaemus*, *L. gununakuna* is territorial; at least in late November/early December, males were observed defending areas in the rocks, making body displays and fighting with neighbors (Avila et al., personal observation). No conclusive evidence of viviparity can be offered, but all related species of the *Liolaemus elongatus-kriegi* complex have this reproductive mode. Juvenile lizards were observed from late March to early May, as in other members of the group.

#### *Liolaemus talampaya* sp. nov.

*Holotype*.—Fundacion Miguel Lillo (FML) 13411 (Fig. 3), an adult male from Rio Las Yeguas, Sierra de los Tarjados, Parque Nacional Talampaya (29° 44' S, 67° 45' W, 1200 m, Fig. 3), Felipe Varela Department, La Rioja Province, Argentina; collected by M. Archangelsky, 8 February 2000.

*Paratypes*.—MLP.S 2400 (male), 2401 (female), FML 13045, 13412 (males), 13413, LJAMM 2684 (females), Rio Las Yeguas, Sierra de los Tarjados, Parque Nacional Talampaya, Felipe Varela Department, La Rioja Province, Argentina, collected by L. J. Avila, M. Morando, and F. Cruz, 28 October 1999.

*Diagnosis*.—*Liolaemus talampaya* is a small and slender member of the *petrophilus* group and can be distinguished from other species by a combination of dorsal coloration and scale count characteristics. *Liolaemus petrophilus* and *L. gununakuna* have a body pattern of transverse dark bars, black ringed tail, basic green background coloration, none of which are ever present in *L. talampaya*. Mid-body scale number in these two is higher than, and nonoverlapping with, that of *L. talampaya* (Table 1). *Liolaemus austromendocinus* has a pale brown dorsal coloration with an indistinct pattern, covered by small scattered irregular flecks. The tail is ringed by indistinct

brown stripes, has weakly keeled dorsal scales, and has a higher number of mid-body scales relative to *L. talampaya*, which has a tan or light brown dorsal coloration, with distinctive lateral black flecks, and a lower number of midbody scales. The group composed of *L. capillitas*, *L. dicktracyi*, *L. heliodermis*, and *L. umbriifer* can be distinguished because these species usually have dark background body coloration not present in *L. talampaya*. Males of *L. heliodermis* also have a distinctive sulfur-yellow dorsal coloration and weakly keeled dorsal scales; *L. dicktracyi* have weakly keeled to keeled dorsal scales, but the body pattern background is indigo/light blue; and *L. umbriifer* also have weakly keeled dorsal scales and a different color pattern. Unlike *L. heliodermis*, *L. dicktracyi*, and *L. umbriifer*, *L. talampaya* have distinctly keeled dorsal scales.

*Description of holotype*.—Adult male (Fig. 3), 85.0 mm SVL; tail length 164.0 mm, complete, nonregenerated. Axilla-groin distance 36.2 mm. Head length 20.1 mm, 16.6 mm wide, 10.2 mm high. Snout length 6.7 mm. Interorbital distance 1.2 mm. Eye-nostril distance 6.1 mm. Orbit-auditory meatus distance 6.1 mm. Orbit-tip of snout distance 8.6 mm. Forelimb length 30.3 mm. Hindlimb length 56.7 mm. Tibial length 19.6 mm. Foot length 25.5 mm. Belly open longitudinally.

Dorsal head scales smooth, 13 between occiput at the level of anterior border of tympanum to rostral, pitted with numerous small scale organs in the snout region. Rostral scale wider (3.7 mm) than high (1.7 mm). Two postrostrals. Nasal scales slightly longer than wide, subtriangular, with oval nostril over one-half length of nasal, lateral, posterior in position. Scales surrounding nasals six on each side; nasal scale in contact with rostral. Six internasals, two larger in middle, separated from nasal by two small scales, in tandem. Four frontonasals, irregular in shape. Five prefrontals, symmetrically arranged, two elongated on sides, two larger, heptagonal, separated by one smaller, subhexagonal in middle. Three anterior frontal scales, smaller than larger prefrontals, symmetrically arranged, followed posteriorly by two azygous frontals and two posterior frontals. Interparietal scale irregular, subpentagonal, with opalescent white "eye", surrounded by five scales: three smaller anteriorly and two almost three times larger

posteriorly. Parietals smooth, flat, irregular. Supraorbital semicircles, 10–10. Circumorbitals 10–10. Five supraoculars transversally expanded on each side, with slightly smaller scales in front and sides, 13 right, 14 left. Posterior canthal scale overlapping anterior superciliary; anterior higher than long. Superciliaries 8–7 (right/left) strongly imbricated, flattened and elongated, anterior six (right side) and five (left side) broadly overlapping dorsally. Two loreal scales. Orbit with 12–14 upper ciliaries, flat and quadrangular, and 15–16 lower ciliaries, rectangular and moderately projecting. Orbit diameter  $3.8 \times 3.2$  mm. Preocular small, unfragmented, longer than wide. Subocular scale elongated, eight times longer than wide ( $6.4 \times 0.8$  mm). Postocular overlapping subocular, almost one-third length of subocular. A longitudinal ridge along upper margin of three ocular scales. Palpebral scales, small, irregular, and flat. One row of lorilabials flat, six on right, eight on left, equal or slightly higher than supralabials, four in contact with subocular. Supralabials four on right, followed by two postlabial scales; five on left, followed by three postlabial scales. Supralabials flat or slightly convex, postlabials and last scale on each side strongly convex, bulging.

Lower temporals imbricate, with a blunt keel; upper temporals juxtaposed, smooth or with small blunt keel. Anterior auriculars smaller than adjacent posterior temporals, slightly tipped in white; same scales slightly projecting. Posterior and lower auriculars, small, almost granular. Auditory meatus rectangular in shape ( $4.1 \times 2.8$  mm). Lateral scales of neck small, irregular, almost granular, non-overlapping, with slightly inflated skin. Antehumeral, gular, longitudinal neck and postauricular folds distinct, oblique neck and antegular less conspicuous, rictal not present. Scales of dorsal neck region similar to body dorsals.

Mental scale in contact with four scales, wider (3.7 mm) than high (2.2 mm). Mental followed posteriorly by two rows of five chinshields. Infralabials flat or slightly convex, four on each side, followed by one small postlabial scale. Gular scales smooth, flat; between chinshields slightly juxtaposed, elliptic, or roughly cylindrical, becoming rounded and imbricate toward auditory meatus. Forty-one gulars between tympana. Infralabials

separated from chinshields by one to three rows of small scales.

Dorsal body scales strongly imbricate, some lanceolated, juxtaposed near limb insertions, with a distinct keel. At midbody, dorsal scales grading to smaller scales on sides, with small blunt keels or almost smooth surface. Scales immediately anterior, above, and posterior to limb insertion, small, almost granular. Ventral body scales quadrangular, smooth, imbricated, slightly larger than dorsals. Sixty-three scales around midbody. Dorsal scales between occiput and anterior margin of hind limb articulations, 66. Ventral scales between mental and precloacal pores, 101. Scales of cloacal region about equal or smaller in size to ventral body scales. Six evident precloacal pores.

Anterior suprabrachials rhomboidal, imbricated, faintly keeled, some slightly larger than dorsal body scales, grading into smooth and rounded posteriorly. Posterior suprabrachials smaller, smooth, becoming granular near axilla. Anterior antibrachials similar to suprabrachial. Posterior antibrachials smaller, smooth, rounded. Supracarpals rounded to rhomboidal, smooth. Infracarpals imbricate, rhomboidal, with a blunt, small keel. Pre- and postdigital scales of manus smooth. Subdigital lamellae with three blunt keels, some terminating in a short mucron; numbering I: 14, II: 22, III: 21, IV: 15, V: 10. Claws robust, curved, clear opaque brown, about similar length of penultimate phalanx. Anterior suprafemorals as large as dorsal body scales, rhomboidal to lanceolate, imbricate, with a small blunt keel, grading posteriorly in small scales, smooth and rounded, almost granular. Supratibials similar to anterior suprafemorals. Supratarsals imbricate, with a weak keel. Postfemorals small, granular, smooth. Infracarapals and infratibials smooth, rounded, imbricate, similar in size to ventral body scales. Infratarsals rounded, smooth, imbricate. Subdigital lamellae on toes: I: 13, II: 16, III: 22, IV: 27, V: 16. Claws robust, curved, opaque brown, about one-third length of penultimate phalanx. Dorsal and lateral caudals strongly keeled, imbricate, some larger than dorsal body scales. Ventral caudals irregular near cloacal opening, rounded, smooth, becoming imbricate in the posterior half of the tail.

*Color in life.*—Head uniformly brown. Background dorsal coloration between occiput

and first caudal annuli light brown, forming a longitudinal wide stripe (Fig. 3). Faded but distinctive incomplete transversal bands dark brown in the lateral and dorsolateral fields, almost indistinct in the neck, becoming more evident in tail forming a “ring” pattern visible only in life. Laterally, a pattern of white, irregular, transversal bands, between neck and groin, some bands spotted but well distinguished between axilla and midbody, intermixed with lateral brown bands. A distinct pattern of small dark black spots spread on sides of body in pre-, supra- and post-scapular areas, reaching midbody, fading caudally to less evident gray spots, reaching groin area. Dorsal areas of limbs tan with irregular, transversal brown bands. Ventral areas light to dark gray, some black areas irregularly distributed; white areas in supra- and infralabials, first gulars, and chinshield scales; a distinctive white line 2–3 scale wide on throat. Ventral limb coloration darker than ventral areas. Bright yellow along ventral femoral and lower ventral scales; intense red scales surrounding cloacal opening. Precloacal pores yellow–orange.

*Color in preservative.*—All background coloration faded, but pattern of lateral black spots remains very evident, as well as white bands; pattern of transversal brown bands fades completely; ventral areas become darker, and all yellow and red coloration disappears.

*Variation in paratypes.*—In three males; SVL (mean  $\pm$  SD [range]):  $81.1 \pm 3.9$  (78.0–85.5). Axilla–groin distance:  $33.0 \pm 1.0$  (32.0–34.0). Head length:  $18.1 \pm 1.7$  (16.7–20.1). Head width:  $15.3 \pm 1.2$  (14.1–16.5). Forelimb length:  $33.6 \pm 1.2$  (32.3–34.7). Hind limb length:  $54.8 \pm 2.8$  (52.8–58.1). Midbody scales: 58–67. Dorsal scales: 64–68. Ventral scales: 91–95. Precloacal pores: 3–5. In three females; SVL (mean  $\pm$  SD [range]):  $74.0 \pm 2.6$  (71.6–76.8). Axilla–groin distance:  $33.0 \pm 1.5$  (31.5–34.6). Head length:  $16.0 \pm 0.4$  (15.7–16.4). Head width:  $13.6 \pm 0.6$  (12.9–14.0). Forelimb length:  $31.7 \pm 0.6$  (31.0–32.1). Hind limb length:  $50.7 \pm 0.5$  (50.4–51.4). Midbody scales: 62–69. Dorsal scales: 64. Ventral scales: 86–96. Precloacal pores not present in females.

Interparietal scale usually irregularly shaped, bordered by 4–7 scales. Supraocular scales: 4–6. Number of scales around nasal

4–5. Nasal completely in contact with rostral (50%) to slightly contact (50%). Scales between rostral and frontal: 5–6. Supralabial scales 6–8. Infralabial scales 5–6. Lorilabials: 6–10. Lorilabials in contact with subocular: 3–4. Fourth finger lamellae counts: 19–22. Fourth toe lamellae counts: 27–30. Gular scales between both auditory meatus: 45–52. Internasal scales 4–5. Upper temporals smooth to slightly keeled, lower temporals smooth. Parietals smooth, flat.

In some lizards, the entire head appears dark brown/gray, turning dark gray in preservative. Limb coloration is dark brown in some individuals, with no spots or marks. Sometimes the dorsolateral pattern of transverse banding extends dorsally almost to the vertebral area between the forelimbs. In some individuals, isolated white spots are irregularly distributed over the dorsal area between the neck and midbody, and, in one individual, white coloration is more extensively distributed on the back of the body and tail, extending to dorsal forelimbs. All lizards observed in the field seem to be light emerald green before capture, but this coloration disappears quickly after capture.

*Sexual dimorphism.*—As in other members of this complex, no body size dimorphism or squamation differences were observed. The base of tail of males is expanded laterally, and the yellow-orange precloacal pores are larger and obvious, as well as the yellow and red coloration observed in femoral, lower ventral, and cloacal areas. Precloacal pores not present in females.

*Etymology.*—Talampaya is the name of the collection area of these lizards, a rock formation of sedimentary origin known for very important fossil discoveries in the last 40 yr. The name Talampaya is a “quichua” (a South American aboriginal language) word meaning “the dry river of the Tala” (Tala is a native tree: *Celtis spinosa*).

*Distribution.*—*Liolaemus talampaya* is known only from the type locality in Sierra de los Tarjados, Talampaya National Park, La Rioja Province, Felipe Varela Department (Fig. 3). It may be expected to occur along the western slope of Sierra de Sañogasta, but suitable habitat similar to the type locality habitat is not very common in this area, and *L. talampaya* is not known in microhabitats other

than the type locality. The known distribution area is part of a sedimentary basin named Ischigualasto–Villa Union Triassic Basin, formed by continental sediments deposited by rivers, lakes, and swamps over the entire Triassic Period. Lizards were collected only in the red sandstone cliffs of the Talampaya and Tarjados geological formations. The plant community is typical of the Monte phytogeographic province, characterized by xeric shrubs of the Zygophyllaceae family, cactus, and some small trees (mainly *Prosopis* spp.) restricted to the edges of ephemeral streams (Femenía and Aceñolaza, 1998).

*Natural history.*—All lizards were observed basking on the cliffs or eroded rocks, but usually retreated to the protection of the rock crevices if an attempt was made at capture. When L. J. Avila and M. Morando visited the type locality in mid–spring and late–summer (October 1999, March 2000), lizards were active during 1000–1900 h. No other *Liolaemus* species were collected in sympatry with *L. talampaya*, but *L. olongasta*, *L. pseudoanomalus*, and *L. darwini* were found in areas surrounding the type locality. Only *Homonota fasciata* was found in close sympatry with *L. talampaya*.

#### MOLECULAR PHYLOGENETIC ANALYSES

With the separate phylogenetic analyses, only few incongruences were found. Within the *kriegi* group, the 12S partition recovered a sister relationship between *L. kriegi* and *Liolaemus* sp. 8 (PP = 0.87); and a clade including these two and *Liolaemus* sp. A and *Liolaemus* sp. B (PP = 0.58); this is not supported with the other MtDNA genes. Within the *elongatus* group, the 12S partition recovered [*L. elongatus* + *Liolaemus* sp. 5] (PP = 0.98) as a monophyletic group, with *Liolaemus* sp. 6 as basal, while the other mitochondrial genes recovered [*L. elongatus* + *Liolaemus* sp. 6 + *Liolaemus* sp. 7] (PP = 0.73 and 0.75 respectively) as monophyletic, with *Liolaemus* sp. 5 as basal. Since most of the nodes did not present conflict among the four gene partitions, all were combined in further analyses. The MP search recovered two most-parsimonious trees (L = 3025, consistency index = 0.475, retention index = 0.575), and a strict consensus tree was generated to compare with the ML, Bayesian, and MetaPiga

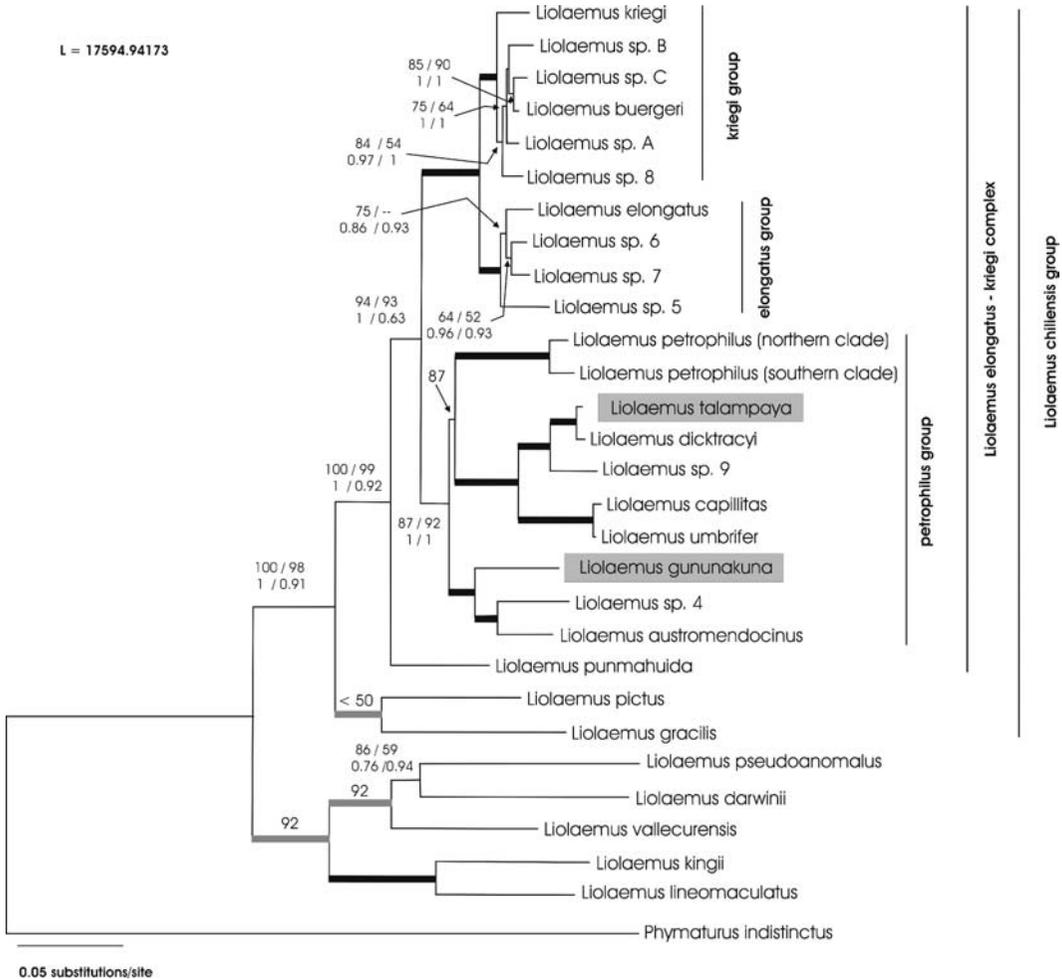


FIG. 4.—Single ML tree (lnL = -17594.94173) of relationships of all samples of the *petrophilus* group included in this study, based on combined *cyt-b*, *ND4*, *12S*, and *C-mos* gene regions. Thicker black branches have MP and ML bootstrap values  $\geq 95\%$ , and posterior probabilities (PP) and genetic algorithm (GA) support values  $\geq 0.95$ . Thicker gray branches have ML bootstrap values  $\geq 95\%$ , and PP and GA support values  $\geq 0.95$ ; variable MP bootstrap values indicated above the branches. For all other internal branches, numbers above represent MP and ML bootstrap values, and numbers below represent PP and GA support values. Terminals identified as *Liolaemus* sp. followed by a number correspond to undescribed species; and those identified as *Liolaemus* sp. followed by a letter, represent candidate species for which more evidence is needed (see Morando et al., 2003).

trees. In the Bayesian analyses, stationarity was reached before 60,000 generations for the independent searches, and the majority consensus tree was obtained from the 14,399 trees remaining after the burn-in. The consensus trees of the two independent analyses recovered identical topologies; this topology was very similar to the strict consensus MP tree. The MetaPiga majority consensus tree also was almost identical to those recovered by the MP and Bayesian methods.

The ML analyses recovered one tree (ln L = -17594.94173) and, because all analyses produced very similar results, the single ML tree is the only one presented here (Fig. 4). Two main clades are recovered with strong support, the first (maximum likelihood bootstrap [ML-B = 100%], maximum parsimony bootstrap [MP-B = 100%], Bayesian posterior probability [PP = 1], genetic algorithm [GA = 1]) includes strongly supported as sister taxa, two species considered basal (*L. kingii* + *L. lineomacula-*

tus), and another clade, also strongly supported with *L. vallecurensis* as basal to the weakly supported clade [*L. pseudoanomalous* + *L. darwini*].

The second main clade recovered (ML-B = 99, MP-B = 100%, PP = 1, GA = 0.91) corresponds to the "chiliensis" group, with [*Liolaemus gracilis* + *L. pictus*] as the sister clade to the strongly supported *Liolaemus elongatus-kriegi* complex (ML-B = 99%, MP-B = 100%, PP = 1, GA = 0.92). In general, the relationships within this complex are the same as obtained in the MP search in Morando et al. (2003), but with higher support values in the relationships where the MP tree was different from the ML/Bayesian trees. The most basal species of this complex is *L. punmahuida* (Avila et al., 2003), with the *petrophilus* group (ML-B = 92%, MP-B = 87%, PP = 1, GA = 1), recovered as the sister taxon (ML-B = 93%, MP-B = 94%, PP = 1, GA = 0.63) of the [*kriegi* + *elongatus*] groups (ML-B = 100%, MP-B = 100%, PP = 1, GA = 1). Within the *kriegi* group, the most basal species is *L. kriegi* and although the relationships between the other terminals are the same of Morando et al. (2003), the species boundaries of these populations still require more extensive study. Within the *elongatus* group, the most basal species is *Liolaemus* sp. 5, with [*Liolaemus* sp. 6 + *Liolaemus* sp. 7] (ML-B = 52%, MP-B = 64%, PP = 0.96, GA = 0.93), as the sister clade of *L. elongatus* (ML-B = <50%, MP-B = 75%, PP = 0.86, GA = 0.93). In the *petrophilus* group one clade includes *L. gununakuna* as the sister species of [*Liolaemus* sp. 4 + *L. austromendocinus*] (ML-B = 96%, MP-B = 98%, PP = 1, GA = 1); the second clade, only supported with MP-B (87%), includes the two different groups identified within *L. petrophilus* in Morando et al. (2003), as the sister species of the strongly supported group (ML-B = 100%, MP-B = 100%, PP = 1, GA = 1) that contains *L. capillitas* + *L. umbrifer* (ML-B = 100%, MP-B = 100%, PP = 1, GA = 1), and *Liolaemus* sp. 9 + (*L. talampaya* + *L. dicktracyi*) (ML-B = 100%, MP-B = 100%, PP = 1, GA = 1).

#### DISCUSSION

During the last 10 yr, taxonomic studies carried out on *Liolaemus* taxa previously

referred to as widespread species have shown the existence of a number of undescribed forms (e.g., Avila, 2003; Cei and Avila, 1998; Cei and Scolaro, 1999; Etheridge, 1992, 1993, 2001; Lobo and Kretzschmar, 1996; Morando et al., 2003), and recent exploration of some poorly known areas of northwestern Patagonia allowed the discovery of several undescribed species (L. J. Avila and M. Morando, unpublished data; M. I. Christie, personal communication; Avila et al., 2003; Etheridge and Christie, 2003; Videla and Cei, 1996). During 1995, herpetological exploration in the middle Neuquén Province resulted in the collection of several undescribed and poorly defined species; one of these was initially considered as the northwesternmost population of the Patagonian Steppe species *Liolaemus petrophilus*, but with a very distinctive coloration (Avila, 1996). This first identification was based on Cei's (1974) findings of a southern population of *L. petrophilus* with "peculiar and brilliant yellow coloration" in northwestern Chubut Province, and on discussion J. M. Cei. However, additional explorations, new samples, and molecular analysis have shown that this population, here named *L. gununakuna*, is not closely related to *L. petrophilus*. *Liolaemus gununakuna* inhabits ecotonal Patagonian Steppes-Austral Monte areas of the western basin of the Limay River, while *L. petrophilus* is restricted to volcanic plateaus of the Patagonian Steppes (Morando et al., 2003). Sampling in western Chubut was carried out, but no "yellow" populations of *L. petrophilus* were ever found.

Farther north of this region, in the complex landscape formed by the northwestern Sierras Pampeanas and other mountain chains parallel to the Andes, a radiation of species closely related to *Liolaemus capillitas* was found (Espinoza and Lobo, 2003; Espinoza et al., 2000), but several new species are still undescribed in this group (L. J. Avila, unpublished data; R. Espinoza, personal communication; F. Lobo, personal communication). While most of these species are found in high elevations (above 2500 m), *L. talampaya* inhabits low elevation areas with a typical Monte vegetation (at approximately 1000 m), but it represents the southernmost member of the group that Espinoza and Lobo (2003) referred as the northern radiation of species

related to *L. capillitas*. Only southern latitude members of the *Liolaemus elongatus-kriegi* complex (800 km south of the distribution area of this new species) are known to inhabit these lower altitudes.

Recent phylogenetic analyses using molecular (Schulte et al., 2000) and morphological (Lobo, 2001) data included some of the species presented in this study. In agreement with our results, both authors found evidence for a sister relationship between *L. austromendocinus* and *Liolaemus* sp. 4 (referred as *L. elongatus* in Schulte et al. [2000] and as *L. cf. elongatus* in Lobo [2001]); one of the new species described here, *L. gununakuna*, is recovered as the sister taxon of this clade (Fig. 4).

In Schulte et al. (2000), *Liolaemus capillitas* is the basal species of a monophyletic group that includes *L. buergeri*, *L. ceii*, *L. leopardinus*, *L. petrophilus*, *L. austromendocinus*, and *Liolaemus* sp. 4; while in Lobo (2001), *L. buergeri* and *L. kriegi* are recovered outside of the species we recovered in the *elongatus* and *petrophilus* groups. Here, in agreement with Morando et al. (2003), we present strong evidence for the monophyly of three groups, *elongatus* as the sister clade of the *kriegi* group, and the *petrophilus* group as the sister clade of these two (Fig. 4).

Within the *petrophilus* group, *Liolaemus talampaya* is the sister species of *L. dicktracyi*, and closely related to *Liolaemus* sp. 9, from the Chaschuil Valley in Catamarca Province. *Liolaemus capillitas* is the sister species of *L. umbrifer*, and probably *L. heliodermis* (not included in this study) is also included in this group (Espinoza et al., 2000). *Liolaemus petrophilus*, distributed approximately 1200 km further south, is weakly recovered as the sister clade of this group. Future work will target unsampled isolates in the northern region of the group's distribution (Fig. 1) and integrate additional nuclear gene regions and morphological data into tests of relationships and species boundaries.

#### RESUMEN

Describimos dos nuevas especies de lagartijas del género *Liolaemus* del oeste de Argentina. Ambas especies pertenecen al grupo *petrophilus* y son fácilmente distinguibles de otros miembros por una combinación

de caracteres de coloración y escamación. Utilizamos secuencias de genes mitocondriales *cyt-b*, 12S, ND4 y del nuclear *C-mos* para inferir la filogenia de las especies incluidas en el grupo. Encontramos evidencia para un grupo *petrophilus* monofilético y basal dentro del complejo *L. elongatus-kriegi*. El grupo *petrophilus* incluye *Liolaemus petrophilus* y dos clados fuertemente soportados, uno contiene las especies distribuidas en el norte, incluyendo una de las especies nuevas, *L. talampaya*; el segundo clado incluye las especies distribuidas en el sur, incluyendo la nueva especie, *L. gununakuna*.

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## APPENDIX I

*Specimens Examined*

*Liolaemus austromendocinus* (63).—ARGENTINA: MENDOZA: Malargüe Department, Portezuelo del Viento, 18 km W, 3.5 km N Bardas Blancas: MVZ 126458–65; 2 km E Agua Botada: MVZ 126480–81; Agua Botada: MVZ 126490–92; Ruta Nacional 40, 2 km N Agua Botada (35° 45' S, 69° 38' W): LJAMM 2717–18, 2740–41; Cuesta El Chihuido: MVZ 126482–83; Mehanquil: MVZ 126478–79; Ruta Provincial 180, 70 km S El Nihuil (35° 39' S, 68° 41' W): LJAMM 4012–16; Ruta Provincial 180, 102 km S El Nihuil (35° 53' S, 68° 37' W): LJAMM 4031–33; Ruta Provincial 180, 22.9 km N Ruta Provincial 186 junction, 3.1 km S Puesto La Ventana (35° 55' S, 68° 36' W): LJAMM 4125–28; Ruta Provincial 180, 116 km S El Nihuil (35° 57' S, 68° 41' W): LJAMM 4199; Ruta Provincial 180, 12 km S Mina Ethel (36° 05' S, 68° 44' W): LJAMM 4000–01; 5 km NE La Salinilla (36° 13' S, 68° 31' W): LJAMM 4143–44; Ruta Nacional 40, 8.3 km S Malargüe (35° 33' S, 69° 35' W): LJAMM 4057–58; Payun Plateau, 22.8 km E Ruta Provincial 183 junction, 13.8 km E junction Puesto El Clavado (36° 39' S, 69° 16' W): LJAMM 4122–23. San Carlos Department: Laguna El Sosneado: MVZ 188641–42; 21 km WNW (by road) El Sosneado; Pampa de los Bayos, 17 km W, 15 km N Cerro Diamante: MVZ 126488; Arroyo Hondo, 20 km W, 17 km N Cerro Diamante: MVZ 126489. San Rafael Department: Junction Ruta 40 and Rio Diamante, 8 km E, 11 km N Cerro La Leña: MVZ 126466–72; 0.5 km S La Juaia, 22 km W, 5 km S Cerro Diamante: MVZ 126484–87. 9.5 km N El Nihuil road to Cañón del Atuel (34° 59' S, 68° 37' W): LJAMM 2715–16; El Nihuil: LJAMM 2722.

*Liolaemus capillitas* (11).—ARGENTINA: CATAMARCA: Andalgalá Department: Ruta Provincial 47, between Km 34 and Km 39: BYU 47100, LJAMM 2766–69, 2786–92.

*Liolaemus dicktracyi* (13).—ARGENTINA: LA RIOJA: Famatina Department: 24 km SW Alto del Carrizal (28° 55' S, 67° 40' W): LJAMM 2106–2107, 5816–5824, 5750–51.

*Liolaemus gununakuna* (19).—ARGENTINA: NEUQUEN: Zapala Department: 2 km SE La Amarga (39° 06' S, 69° 34' W): FML 12717 (holotype), 12720, MLP.S 2352–53, LJAMM 2690 (paratypes); Los Candeleros, SE Cerro Lotena, 45 km SW Cutral C6: BYU 47309–11, FML 12719, LJAMM 287, 2690 (paratypes); rocky hills near La Amarga: FML 12718, LJAMM 2438 (paratypes); Bosque Petrificado: FML 13043–44, LJAMM 143, 265–6, 279, 2440 (paratypes).

*Liolaemus petrophilus* (63).—ARGENTINA: RIO NEGRO: El Cuy Department: El Cuy (39° 55' S, 68° 20' W): LJAMM 1612, 1761–65, 1842–47, BYU 47098, FML 8574–75. 25 de Mayo Department: Ruta Provincial 8, 17 km S San Antonio del Cuy (40° 17' S, 68° 27' W): LJAMM 1652; 7.5 km W Los Menucos (40° 51' S, 68° 10' W): LJAMM (fn 88, 90–93); Ruta Provincial 5, 40 km SE Maquinchao (41° 30' S, 68° 33' W): LJAMM (fn 166, 171), BYU 46791; 7 km N Ingeniero Jacobacci (41° 13' S, 69° 24' W): LJAMM 182–7, BYU 46792–93; Ruta Provincial 76, 57 km S Ingeniero Jacobacci (41° 45' 08" S, 69° 21' 34" W): LJAMM (fn 213–4). 9 de Julio Department: Ruta Provincial 66, 18 km NW Comicó (41° 00' S, 67° 40' W): LJAMM (fn 103–109). Ñorquinco Department: Ruta Provincial 6, 1 km NW Ojo de Agua (41° 32' S, 69° 51' W): LJAMM 2138, 2269. Valcheta Department: 1 km W

Chipauquil (40° 58' S, 66° 39' W): LJAMM (fn 51–57). Meseta de Somuncurá (41° 10' S, 66° 51' W): LJAMM 4451. CHUBUT: Langueño Department: Ruta Provincial 12, 8 km S Paso del Sapo (42° 48' S, 69° 33' W): BYU 47097, FML 13063. Gastre Department: Ruta Provincial 12, 65 km S Paso del Sapo (43° 12' S, 69° 12' W): BYU 47094. Paso de Indios Department: Valle de los Mártires, Ruta Nacional 25, Km 249, 19 km E junction Ruta Provincial 27 (43° 49' S, 67° 45' W): LJAMM 2125; Ruta Provincial 12, 6 km N Cerro Condor, 72 km N Paso de Indios (43° 23' S, 69° 10' W): LJAMM (fn 231–234).

*Liolaemus talampaya* (7).—ARGENTINA: LA RIOJA: Felipe Varela Department: Rio Las Yeguas, Sierra de Los Tarjados, Parque Nacional Talampaya (29° 44' S, 67° 45' W): FML 13411 (holotype), MLP.S 2400–1, FML 13045, 13412–3, LJAMM 2684 (paratypes).

*Liolaemus umbrifer* (14).—ARGENTINA: CATAMARCA: Belen Department: Quebrada de Randolpho (26° 51' S, 66° 44' W): LJAMM 5022–33.

## APPENDIX II

*Specimens Used for Molecular Analyses*

Kriegi group.—*Liolaemus buergeri*: ARGENTINA: MENDOZA: Malargüe Department: Mallines Colgados, between Arroyo El Leon and Rio Grande: LJAMM 2744.

*Liolaemus kriegi*: ARGENTINA: RIO NEGRO: 25 de Mayo Department: Ruta Provincial 5, 40 km S Maquinchao: LJAMM 3045. *Liolaemus* sp. A: ARGENTINA: NEUQUEN: Ñorquin Department: Ruta Provincial 26, 5 km E Caviahue: LJAMM 2533. *Liolaemus* sp. B: ARGENTINA: MENDOZA: Malargüe Department: Ruta Nacional 40, 5 km N Ranquil Norte: LJAMM 2444 (cyt b, ND4), 2667 (12S), 2442 (C–mos). *Liolaemus* sp. C: ARGENTINA: NEUQUEN: Chos Malal Department: Ruta Provincial 37, 15 km N Los Barros: LJAMM 2614, 2616 (cyt–b). *Liolaemus* sp. 8: ARGENTINA: NEUQUEN: Ñorquin Department: W Termas de Copahue: SDSU 3695. *Elongatus* group: *Liolaemus elongatus*: ARGENTINA: CHUBUT: Futaleufú Department: Ruta Nacional 40, Km 1530, 17 km S Esquel: LJAMM 2128. *Liolaemus* sp. 5: ARGENTINA: MENDOZA: Malargüe Department: 16 km W Las Leñas, road to Valle Hermoso: FML 13057, LJAMM 2681 (12S, C–mos). *Liolaemus* sp. 6: ARGENTINA: NEUQUEN: Ñorquin Department: Ruta Provincial 26, 5 km E Caviahue: LJAMM 2454, 2523 (12S, C–mos). *Liolaemus* sp. 7: ARGENTINA: NEUQUEN: Chos Malal Department: Ruta Provincial 37, 15 km N Los Barros: LJAMM 2602.

*Petrophilus* group.—*Liolaemus petrophilus*: ARGENTINA: CHUBUT: Telsen Department: Ruta Provincial 8, Sierra Colorada: LJAMM (fn)362. RIO NEGRO: El Cuy Department: El Cuy: BYU 47098. *Liolaemus umbrifer*: ARGENTINA: CATAMARCA: Belen Department: Quebrada de Randolpho: LJAMM 5029. *Liolaemus dicktracyi*: ARGENTINA: LA RIOJA: Famatina Department: 24 km SE Alto del Carrizal, road to La Mejicana: LJAMM 5750. *Liolaemus capillitas*: ARGENTINA: CATAMARCA: Andalgalá Department: Ruta Provincial 47, between Km 34 and Km 39: BYU 47100, LJAMM 2787 (C–mos). *Liolaemus austromendocinus*: ARGENTINA: MENDOZA: Malargüe Department: Ruta Provincial 180, 12.5 km S La Matancilla: LJAMM 5147, 4014 (C–mos). *Liolaemus talampaya*: ARGENTINA: LA RIOJA: Felipe Varela

Department: Rio Las Yeguas, Sierra de Los Tarjados, Talampaya National Park: FML 13412. *Liolaemus gunnisoni*: ARGENTINA: NEUQUEN: Zapala Department: 2 km SE La Amarga: LJAMM 2690. *Liolaemus* sp. 4: ARGENTINA: MENDOZA: Las Heras Department: Vallecitos: BYU 47106, LJAMM 2743 (ND4), 2704 (12S). *Liolaemus* sp. 9: ARGENTINA: CATAMARCA: Tinogasta Department: Ruta Nacional 60, 20 km E Chaschuil, Quebrada Las Angosturas: LJAMM 4227, 4219 (ND4). *Liolaemus punmahuida*: ARGENTINA: NEUQUEN: Chos Malal Department: Tromen Volcano: FML 11958. Outgroups: *Liolaemus pictus*: ARGENTINA: RIO NEGRO: Bariloche Department: Bariloche: BYU 47193. *Liolaemus gracilis*: ARGENTINA: NEUQUEN: Zapala Department: 6 km NW La Amarga: LJAMM 2640.

*Liolaemus pseudoanomalus*: ARGENTINA: LA RIOJA: Felipe Varela Department: Ruta Provincial 26, 3 km N Pagacillo: LJAMM 2300. *Liolaemus darwini*: ARGENTINA: RIO NEGRO: General Roca Department: 18 km NE Villa Regina: LJAMM 2410, 2409 (C-mos). *Liolaemus vallecurensis*: ARGENTINA: SAN JUAN: Iglesia Department: Valle del Cura: LJAMM 2698. *Liolaemus kingii*: ARGENTINA: CHUBUT: Rio Senguer Department: Ruta Nacional 40, 2 km S Rio Mayo: BYU 46777. *Liolaemus lineomaculatus*: ARGENTINA: RIO NEGRO: Bariloche Department: Parque Nacional Nahuel Huapi, NW slope Piedra del Condor, Cerro Catedral: SDSU 4268. *Phymaturus indistictus*: ARGENTINA: CHUBUT: Rio Senguer Department: Ruta Provincial 20, Sierra de San Bernardo: LJAMM 2124.

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## PRIORITY USE OF CHEMICAL OVER VISUAL CUES FOR DETECTION OF PREDATORS BY GRAYBELLY SALAMANDERS, *EURYCEA MULTIPLICATA GRISEOGASTER*

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**ABSTRACT:** Many aquatic amphibians live in habitats with low visibility. In such habitats, chemical cues may be more reliable than visual cues for predator recognition. Adult perrenibranchiate graybelly salamanders, *Eurycea multiplicata griseogaster*, occupy clear-water streams with low levels of sedimentation and relatively few visual obstructions. In a previous laboratory experiment, graybelly salamanders distinguished between chemical stimuli from predatory fish (banded sculpins, *Cottus carolinae*) and nonpredatory tadpoles (*Rana sphenoccephala*). In the present study, when only visual cues were available, salamanders did not distinguish between sculpins and tadpoles. Instead, they reduced activity in response to both predatory and nonpredatory heterospecifics in comparison to a blank control, indicating an alarm response to general disturbance rather than recognition of the specific predator, per se. To confirm that chemical stimuli are important under natural conditions, we tested whether graybelly salamanders in a natural stream habitat distinguished between chemical stimuli from sculpins, nonpredatory fish (stonerollers, *Campostoma pullum*), and a blank control. In contrast to their response to the nonpredator treatments, salamanders quickly moved away from the sculpin stimulus and then burrowed into the gravel substrate. Therefore, even for salamanders from clear-water habitats, chemical stimuli are more effective than visual stimuli for recognition of visually cryptic predators.

**Key words:** Antipredator behavior; Chemical cues; *Eurycea multiplicata griseogaster*; Graybelly salamander; Kairomones; Visual cues

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EVASION of predators is most successful when prey detect predators early in the predation sequence (Lima and Dill, 1990). For aquatic amphibians, chemical cues often function more effectively for predator de-

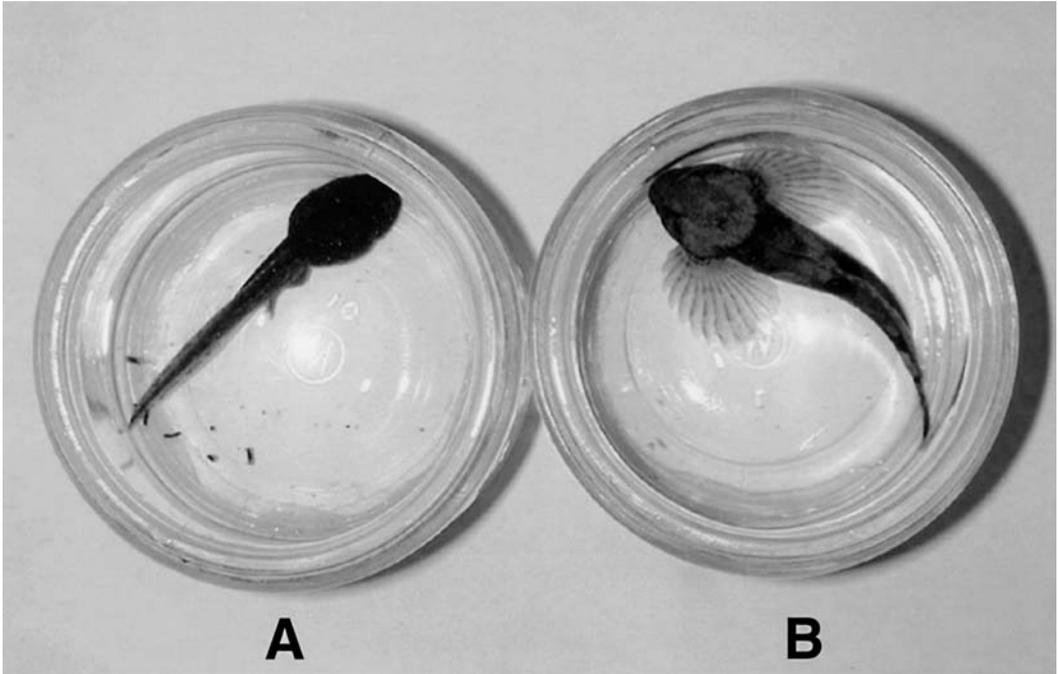


FIG. 1.—Stimulus animals used in the visual experiment: (A) southern leopard frog (*Rana sphenosephala*) tadpole (nonpredator) and (B) banded sculpin (*Cottus caroliniae*) (predator). Note similar size and shape.

tection than visual cues (Kiesecker et al., 1996; Mathis and Vincent, 2000; Stauffer and Semlitsch, 1993). Hypotheses that explain the priority use of chemical over visual cues include the difficulty of detecting cryptic predators, occupation of low-visibility habitats (highly sedimented or vegetated), or developmental constraints leading to poorly developed visual systems (Mathis and Vincent, 2000). In this study, we tested the first of these hypotheses by examining whether chemical cues may be more important than visual cues in predator recognition for aquatic salamanders from clear-water habitats with few visual obstructions.

In the Ozarks region of the United States, some populations of graybelly salamanders, *Eurycea multiplicata griseogaster*, are non-transforming (i.e., perrenibranchiate). Adults occupy streams that are relatively shallow and have clear water and deep gravel substrates (Dundee, 1958; Rudolph, 1978). Because salamanders are active on the stream bottom, they are vulnerable to predation by benthic fishes such as sculpins (*Cottus*). Sculpins are euryphagic feeders (Pflieger, 1997) and readily

consume graybelly salamanders in captivity (N. Nelson, personal communication).

In a previous laboratory experiment (Whitham and Mathis, 2000), graybelly salamanders were shown to use chemical stimuli to distinguish between predatory (sculpin) and nonpredatory (tadpoles, nonpredatory fish) heterospecifics by increasing foraging latency when exposed to the predatory stimuli. We used a similar protocol to determine whether salamanders can distinguish between predatory and nonpredatory heterospecifics using visual cues in the absence of chemical cues. We chose tadpoles for the nonpredatory stimulus because they were similar in size and general body shape to sculpins (Fig. 1).

In some cases, antipredatory responses to chemical stimuli may be magnified under artificial conditions (Irving and Magurran, 1997). We also performed a field experiment to confirm the importance of chemical cues in predator detection for salamanders in natural habitats. Studies of response to chemical cues by prey under natural conditions are relatively rare (e.g., Kats et al., 1988; Mathis et al., 2003; Sullivan et al., 2002). We used sculpins as

predators and herbivorous fish (stonerollers, *Campostoma pullum*) as nonpredatory stimuli in this experiment because they should provide a powerful test of the specificity of the discrimination ability of salamanders (i.e., predatory versus nonpredatory fishes). Whitham and Mathis (2000) found that salamanders responded to the sculpin stimulus with reduced foraging activity. Foraging is difficult to observe under natural conditions, so we used movement as a response variable in this experiment. In our field experiment, we predicted that graybelly salamanders would show reduced movement in response to the predatory sculpin stimulus but not to the nonpredatory stimuli.

#### EXPERIMENT 1: USE OF VISUAL CUES IN LABORATORY TRIALS

##### *Methods*

Aquatic adult *E. m. griseogaster* (mean  $\pm$  1 SD: snout-vent length (SVL) = 33.08  $\pm$  4.33 mm;  $n$  = 120) were collected in Christian County, Missouri. Most (about 90%) individuals were captured in summer and fall 2001, but a few were caught in fall 2000 and spring 2001. Laboratory assays were performed in March 2002, and salamanders were then released. Although there was a relatively long period between capture and testing for some individuals, alarm responses to predatory chemical stimuli for salamanders of this specific population have been observed after several months in captivity (D. Rippe toe and A. Mathis, unpublished data). Assays tested visual stimuli from: (1) predatory sculpins, *Cottus carolinae*, (2) nonpredatory herbivorous tadpoles, *Rana sphenoccephala*, and (3) no visual stimulus (blank control).

Stimulus tadpoles and sculpins were collected in Webster and Christian Counties in Missouri and maintained in the laboratory in monospecific groups in 4-l aquaria on a 14L:10D cycle at 19–22 C. Tadpoles were fed a diet of commercial spirulina discs, and sculpins were fed blackworms (*Lumbriculus variegatus*) ad libitum.

Salamanders were housed individually in plastic containers (12  $\times$  12  $\times$  12 cm) filled with approximately 200 ml of dechlorinated tap water. The water was changed and salamanders were fed blackworms approximately every 4 d. Salamanders were not fed for 4 d prior to

testing to standardize hunger levels because Whitham and Mathis (2000) reported that hunger levels could influence response of graybelly salamanders to predatory stimuli.

Although salamanders in this population are most active at night, individuals often can be observed active on the substrate throughout the day (C. Hickman, personal observation). We conducted this experiment during the day portion of the light cycle because visual cues should be most useful during this period. We always fed the salamanders during the day so that they would be habituated to foraging under light conditions. Moreover, graybelly salamanders in the study of Whitham and Mathis (2000) responded to chemical cues from predators when tested in the laboratory during the day.

Assays were conducted in 4-l aquaria filled with approximately 1 l of dechlorinated water. Treatments were randomly assigned, and aquaria were thoroughly cleaned prior to each assay. Stimulus animals (sculpins and tadpoles) were introduced into the testing tank inside a 266-ml glass jar, which was surrounded with a removable internal opaque barrier. The barrier was internal to the jar, so that any disturbance to the water would affect the stimulus animal rather than the test salamander. The water levels were below the rim of the stimulus jars so that there was no exchange of water between the stimulus jar and the testing tank. Test salamanders were placed inside each testing tank and allowed to acclimate for 30 min. A single stimulus animal was then added to the glass jar; stimulus animals were not added earlier to minimize the time that they had to remain in the relatively small jars. A total of four sculpins (mean  $\pm$  1 SD: total length (TL) = 7.23 cm  $\pm$  0.88) and five tadpoles (mean  $\pm$  1 SD: total length (TL) = 6.36 cm  $\pm$  0.46) were used in the experiment. Tadpoles and sculpins did not vary significantly in total length (Two-sample  $t$ -test,  $t$  = -1.93,  $P$  = 0.10).

After 4 min, the barrier was carefully removed from around the jars and the salamanders were allowed to acclimate for 3 min to the visual presence of their assigned stimulus. A single blackworm was added approximately 3 cm from the snout of the salamander, which required the salamander to move in order to catch the prey. The response variable was the latency to strike the worm.

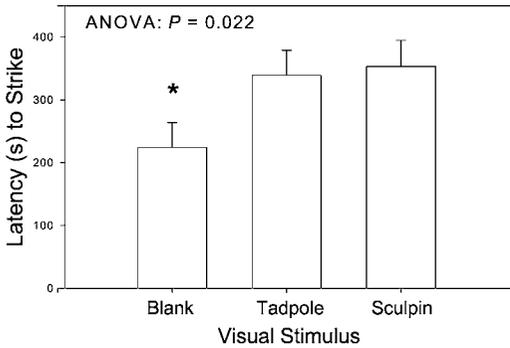


FIG. 2.—Latency (mean  $\pm$  1 SE) to strike prey (blackworms) for salamanders ( $n = 40$  per treatment) exposed to visual cues from: a blank control, nonpredatory tadpoles, and predatory sculpins. \*indicates that the response was statistically different according to non-parametric multiple comparison of treatment and control stimuli.

Timing began when the worm rested on the bottom of the aquarium. A maximum latency score of 600 s was recorded for focal salamanders that did not strike. We tested 40 individuals in each treatment, and salamanders were tested only once.

Because the latency data deviated significantly from normality, we compared latencies among treatments using a Kruskal-Wallis One-Way ANOVA, followed by nonparametric Dunnnett-type multiple comparison tests for equal group sizes between the blank control and stimulus treatments (Zar, 1984).

### Results

There was a significant difference in latency to strike among treatments ( $H = 7.66$ ,  $P = 0.022$ ) (Fig. 2). Latency to strike was shorter in the blank treatment than either the tadpole ( $q = 3.46$ ,  $P < 0.01$ ) or the sculpin ( $q = 2.32$ ,  $P < 0.05$ ) treatments.

#### EXPERIMENT 2: USE OF CHEMICAL CUES UNDER NATURAL CONDITIONS

##### Methods

Field assays were conducted between mid-September and mid-October 2001 in Christian County, Missouri. Assays compared responses to chemical stimuli from: (1) predatory banded sculpins, *C. carolinae*, (2) herbivorous non-predatory stonerollers, *Campestris pullum*, and (3) a water control of dechlorinated tap water (hereafter, "blank").

We collected sculpins and stonerollers in Christian County, Missouri, and held them in the laboratory in 38-l aquaria on a 14L:10D cycle at 19–22 C. Stonerollers were fed a maintenance diet of commercial spirulina discs, and sculpins were fed blackworms (*L. variegates*) ad libitum.

Stonerollers and sculpins were fed just prior to placement into filtered and aerated stimulus collection aquaria, but were not fed during the stimulus collection period. Water levels were adjusted so there was approximately 1.9 ml of water per gram of stimulus animal (mean  $\pm$  1 SD: sculpins,  $9.6 \pm 4.55$  g,  $n = 4$ ; stonerollers,  $9.1 \pm 2.24$  g,  $n = 5$ ). This concentration was similar to that used in field study of Mathis et al. (2003) of predator recognition by larval ringed salamanders, *Ambystoma annulatum*. A third aquarium contained the blank treatment with 50 l of filtered and aerated dechlorinated tap water. After 96 h, we removed the stimulus animals, stirred the water, and poured the stimulus water from aquaria into ice trays. Ice trays were wrapped immediately with cellophane and placed in a  $-20$  C freezer for at least 24 h prior to testing. Although there was a chance that freezing the stimulus could lead to chemical alteration of active components, this technique has been used successfully in other studies (e.g., Mathis et al., 1993).

Ozark streams typically vary seasonally in depth, sometimes drying completely during late summer. Aquatic salamanders survive by burrowing into the gravel bed and following the water table (Dundee, 1958). We initially attempted to make observations during early summer, but salamanders were disturbed when we waded in the stream to locate focal animals. We could not make observations from the stream banks, as has been done in other studies (e.g., Mathis et al., 2003) because there was insufficient salamander activity along the stream edge. At the end of summer, the stream became intermittent. We solved the disturbance problem by excavating holes ( $105 \pm 10$  cm width, 20–30 cm depth) in the stream bed until we reached the water table (5–15 cm water depth) and observed salamanders from around the edges of the holes without disturbing the substrate. The intermittence of the stream also prevented contamination of any local predator presence during experimental observations.

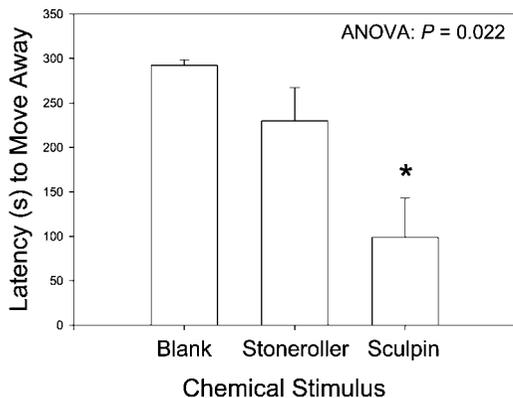


FIG. 3.—Latency (mean  $\pm$  1 SE) to move 21 cm by salamanders exposed to chemical stimuli from: a blank control ( $n = 8$ ), nonpredatory stonerollers ( $n = 9$ ), and predatory sculpins ( $n = 10$ ). \* indicates that the response was statistically different according to nonparametric multiple comparison tests.

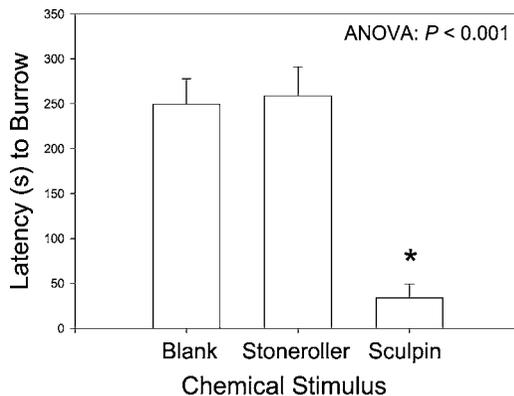


FIG. 4.—Latency (mean  $\pm$  1 SE) to complete a burrow following exposure to chemical stimuli from: a blank control ( $n = 8$ ), nonpredatory stonerollers ( $n = 9$ ), and predatory sculpins ( $n = 10$ ). \* indicates that the response was statistically different according to nonparametric multiple comparison tests.

Holes were spaced approximately 1 m apart. One salamander was tested per hole per night for a maximum of three salamanders per hole; no hole had the same stimulus tested twice. There was a minimum of 24 h between trials at the same hole, and stimuli were randomly assigned to holes each evening. We do not know the extent to which individuals may have moved through the gravel substrate between holes; however, because of the high density of salamanders at this site, we feel that it is unlikely that any individual was tested more than once. Although we do not have any quantitative density estimates, we have collected dozens of individuals within a few square meters of the stream bed.

We constructed a stimulus injection apparatus (SIA) to ensure that each stimulus was accurately introduced at a standard depth and distance from salamanders. The SIA was a 60-ml syringe connected to polyethylene tubing (105 cm) attached to a bamboo splint.

Stimulus ice was thawed at the study site and introduced into the SIA. During each trial, individual salamanders were exposed to 50 ml of a randomly chosen stimulus (sculpin, stoneroller, or blank). The stimulus solutions were coded so that observations were performed blind.

In aquatic habitats, diffusion rates vary unpredictably with currents, structures, or local temperatures. We added 1 drop of blue

food coloring to each thawed stimulus aliquot so that we could observe the stimulus solution come in contact with the snout of the test animal. The food coloring did not appear to disturb normal feeding behavior of these salamanders under laboratory conditions (C. Hickman, personal observation).

Tests were conducted after dusk and no later than 2400 h because salamanders were most active during this period (C. Hickman, personal observation). Water temperatures ranged from 9–17 C. For illumination, we used flashlights covered with red cellophane to reduce the intensity of the light. We located individual test animals within each test hole and carefully lowered the end of the SIA into the water approximately 20 cm in front of the focal animal. The stimulus water was injected at a rate of about 1 ml/s. Salamanders were relatively active and tended to make slow, continuous movements while foraging along the substrate. We quantified activity as latency to move a linear distance of 21 cm. We chose this distance because it was easy to quantify (approximately one-third of the distance across the excavated hole) and because it was a reasonable distance estimate of “typical” movements during preliminary observations of disturbed and undisturbed salamanders. Below we refer to this behavior as latency to “move away.” A maximum latency score of 300 s was recorded for focal animals that did not

move away. Because increased use of refuges is also a common response to predatory stimuli (Kats et al., 1988; Petranka et al., 1987), we also measured latency to burrow completely into the substrate. When burrowing occurred, it generally followed moving away, but sometimes burrowing occurred at a closer distance than 21 cm. If no burrowing occurred, we recorded a maximum latency score of 300 s.

We tested salamanders in each treatment, blank ( $n = 8$ ), nonpredatory stonerollers ( $n = 9$ ), and predatory sculpins ( $n = 10$ ), and compared latencies among treatments using a Kruskal-Wallis One-Way Analysis of Variance, followed by nonparametric Dunn's-type multiple comparison tests for unequal group sizes (Zar, 1984).

### Results

There was a significant difference in latency to move away among treatments ( $H = 7.59$ ,  $P = 0.022$ ; Fig. 3). Latency to move away was faster in the sculpin treatment than in either the blank ( $Q = 15.21$ ,  $P < 0.001$ ) or the stoneroller ( $Q = 16.00$ ,  $P < 0.001$ ) treatments. There was no significant difference in latency to move away between the stoneroller and blank treatments ( $Q = 0.29$ ,  $P > 0.50$ ). Salamanders also exhibited a significant difference in latency to burrow among treatments ( $H = 17.30$ ,  $P < 0.001$ ) (Fig. 4). Latency to burrow was faster in the sculpin treatment than in either the blank ( $Q = 6.53$ ,  $P < 0.001$ ) or the stoneroller ( $Q = 9.06$ ,  $P < 0.001$ ) treatments. There was no significant difference in latency to burrow between the stoneroller and blank treatments ( $Q = 1.92$ ,  $P > 0.20$ ).

### DISCUSSION

When only visual cues were present, graybelly salamanders were disturbed by the presence of both predatory (sculpin) and nonpredatory (tadpole) heterospecifics, but did not discriminate between the two stimuli. This result is in marked contrast to that of Whitham and Mathis (2000), who used a similar experimental protocol and found that graybelly salamanders were able to discriminate between these same stimuli (sculpins and tadpoles) when only chemical cues were present. Priority use of chemical over visual cues for predator detection has been reported for other

aquatic amphibians, including anuran tadpoles (Kiesecker et al., 1996; Stauffer and Semlitsch, 1993) and larval newts, *Notophthalmus viridescens* (Mathis and Vincent, 2000).

Although graybelly salamanders did not distinguish between predatory and nonpredatory categories of visual stimuli, they were disturbed by both in comparison to the blank control, which is identical to responses reported for larval newts (Mathis and Vincent, 2000). General disturbance also may play a role in response of western toad tadpoles (*Bufo boreas*) to visual stimuli (Kiesecker et al., 1996). Although the tadpoles in that study reduced their activity in response to the sight of garter snakes (*Thamnophis sirtalis*), the authors suggested that this response was due to high levels of snake activity rather than to visual cues, per se. An alarm response to any visual disturbance has obvious survival value, but carries the potential cost of reduced fitness due to time lost for foraging or other activities (e.g., mate searching).

The lack of fine-scale discrimination of visual stimuli in this study cannot be explained by a low-visibility habitat, because these salamanders occupy clear-water streams that only rarely become turbid. Even in clear habitats, chemical cues could offer more reliable information for cryptic predators, such as the sculpins used in this study. Moreover, the alarm response to tadpoles possibly could be explained by their similar size and body shape to sculpins. Visual cues may be more important for less cryptic predators or to rule out nonpredators that do not closely resemble potential predators. However, this latter hypothesis does not explain the failure of larval newts to distinguish between predatory and nonpredatory heterospecifics (Mathis and Vincent, 2000) because the two were quite dissimilar in appearance (predatory larval tiger salamanders, *Ambystoma tigrinum*, versus gray treefrog tadpoles, *Hyla chrysoscelis/versicolor*). Other explanations for primary reliance on chemical cues in predator recognition include the ability to detect the presence of predators in the dark (Downes and Shine, 1998) or myopia of the prey (e.g., Mathis et al., 1988).

Salamanders were able to use chemical stimuli to distinguish between predators and nonpredators in a natural stream habitat.

However, in contrast to our original prediction, the nature of the response to the predatory threat was different than in the laboratory study of Whitham and Mathis (2000). Instead of reduced activity, salamanders responded by rapidly fleeing the area, often moving over 21 cm in <1 s, and then burrowing into the substrate. Unlike the study of Irving and Magurran (1997), where responses of minnows, *Phoxinus phoxinus*, appeared muted under natural conditions, the responses of salamanders in our study were more dramatic under natural conditions than in the laboratory.

Although our field study resulted in responses that were different from those in the previous laboratory study, this difference is not necessarily typical. For example, larval ringed salamanders, *Ambystoma annulatum*, responded with reduced activity to chemical stimuli from predatory newts, *Notophthalmus viridescens*, in both laboratory chambers and in a natural pond habitat (Mathis et al., 2003). For graybelly salamanders, reduced activity may be a less preferred antipredator tactic that occurred in the laboratory (Whitham and Mathis, 2000) only because flight or burrowing was not an option in the small enclosed testing chambers. Thus, antipredator responses to ambush predators appear to include a hierarchy of tactics for this species. Under natural conditions, the initial response is rapid flight, followed by burrowing into the substrate. If neither of these responses is practical, as in simplified laboratory habitats, salamanders switch to the less preferred tactic of decreased activity, which may make prey less likely to be detected (Lefcort, 1996).

Why is flight a preferred antipredator tactic of graybelly salamanders, but not of others (e.g., larval *A. annulatum* salamanders)? We suggest three possible answers to this question. First, the foraging strategy of the predator may dictate the best antipredator response. Ambush predators are unlikely to pursue fleeing prey (Keenleyside, 1979), but flight may be ineffective against predators that rely on movement for prey detection. Second, visibility in the habitat can influence whether or not nonmoving prey are likely to escape detection by predators. Reduced activity may be beneficial for avoiding detection by predators in habitats where visibility is restricted but would

provide less protection in clear habitats. Third, flight may be a more effective antipredator tactic for individuals whose body shape promotes rapid swimming speed. Decreased activity may be more effective than flight for larval ringed salamanders because they have active, visual predators (newts: Attar and Maly, 1980; Martin et al., 1974), occupy ponds with high levels of sediments and vegetation, and have relatively shortened bodies. In contrast, decreased activity should offer minimal protection for graybelly salamanders in our study because they have ambush predators (sculpins), occupy clear-water habitats, and have highly streamlined bodies. Depending on testing conditions, antipredator responses of graybelly salamanders appear to follow a hierarchy of tactics, ranging from flight to burrowing to reduction of activity.

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