Journal of Chemical Ecology, Vol. 27, No. 8, 2001

CHEMICAL COMPOSITION OF PRECLOACAL SECRETIONS OF *Liolaemus* LIZARDS

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(Received October 24, 2000; accepted April 16, 2001)

Abstract—Interspecific chemical variation of precloacal pore secretions of *Liolaemus* lizards was characterized in 20 species, and intraspecific chemical variation was characterized using nine individuals of *L. bellii*. The latitude (Chile, 18° to 33° South latitude) and altitude (100 to 4350 m.a.s.l.) of the capture sites were recorded, as well as the number of precloacal pores of each lizard. Secretions were analyzed by GC-MS. A total of 49 compounds were found distributed among the 20 species of *Liolaemus*. Different chemical patterns occurred at intra- and interspecific levels. Compounds belonged to three main families: *n*-alkanes, long chain carboxylic acids, and steroids. Cholesterol and five carboxylic acids (tetradecanoic, hexadecanoic, hexadecenoic, octadecanoic, and *Z*-9-octadecenoic) appeared in all species. The number of precloacal pores correlated positively with altitude and negatively with latitude, suggesting that lizards produce more secretions under harsh environments.

Key Words—Chilean lizards, Tropiduridae, *Liolaemus*, precloacal pores, holocrine glands.

INTRODUCTION

Chemical communication is a well-documented phenomenon in different lizard genera (for reviews, see Mason, 1992; Font, 1996; Cooper, 1998). Pheromones are important for territory marking (e.g., Alberts, 1992), discrimination of familiar from unfamiliar individuals (e.g., Cooper, 1996), self-recognition (Graves and Halpern, 1991), and sexual recognition (Cooper et al., 1996). Although *Liolaemus* is a lizard genus with more than 150 species (Etheridge, 1995), little is known

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0098-0331/01/0800-1677$19.50/0

about the pheromones and chemical communication of its species. Chemical self-recognition and conspecific discrimination have been shown in *L. tenuis* (Labra and Niemeyer, 1999) and *L. bellii* (Labra et al., 2001). Potential sites of semiochemical production are the cloaca and the precloacal pores, the latter located in the external border of the cloacal shield and present mainly in males in the genus *Liolaemus* (Donoso-Barros, 1966). Given that precloacal pores have been described as producing secretions in lizards (Chauhan, 1986a, 1986b), and amphisbaenians (Antoniazzi et al., 1993, 1994), and that these secretions have pheromonal properties (Cooper et al., 1994; López et al., 1997), it is likely that precloacal secretions in *Liolaemus* also are involved in chemical communication. In fact, *Liolaemus* males are often seen dragging their cloaca over the substrate (Labra, personal observations), as has been observed in amphisbaenians (Chauhan, 1986b), a behavior allowing the release of secretions.

Proteins and lipids are the main compounds involved in chemical communication of squamates, although it is not clear which particular chemical compounds (or combination of them) are most relevant in this process. Although inter- and intraspecific variation in the protein content of femoral glands are claimed to be responsible for specific, self, and sex chemical recognition in different lizard species (Alberts, 1991; Alberts et al., 1993), most evidence points to lipids in pheromonal secretions as the main compounds involved in communication. The lipid content in the secretion of femoral glands (Alberts et al., 1992) and skin secretions (Mason and Gutzke, 1990) has been extensively studied, and *n*-alkanes, long chain carboxylic acids, and cholesterol and its derivatives have been found (Alberts, 1990; Weldon et al., 1990; Alberts et al., 1992). Bull et al. (1999) proposed that discrimination of individuals is determined by a complex relationship among different lipidic compounds found in feces, and Cooper and Garstka (1987) found that neutral lipid fractions of urodaeal glands secretions had pheronomal properties.

We report herein a characterization of the lipidic fraction of precloacal secretions of 20 *Liolaemus* species from different localities of Chile and from nine individuals of *L. bellii*, in order to evaluate the chemical patterns at the species and individual levels. The potential effect of environmental factors on the chemical composition of secretions was evaluated.

METHODS AND MATERIALS

Sample Collection. Liolaemus males were captured between October 1999 and February 2000, in northern and central Chile (Table 1). To collect secretions, males were placed backwards and pores were pressed gently with forceps. The yellow-reddish greasy secretion of each individual was dissolved in 400 μl purified *n*-hexane (see below), and placed individually in glass ampoules, which were

TABLE 1. MEAN NUMBER OF PRECLOACAL PORES (NP) OF 20 *Liolaemus* SPECIES, STANDARD ERRORS (SE), RANGE OF NUMBER OF PORES RECORDED, AND SAMPLE SIZE (*N*). CAPTURE SITES OF THE SPECIES INCLUDE THE ALTITUDE (M.A.S.L.) AND THE LATITUDE/LONGITUDE (°S/°W)

	Pores				Capture sites	
Liolaemus	np	SE	Range	*N*	Altitude	Latitude/Longitude
alticolor	2.75	0.48	2–4	4	4350	18°10'; 69°25'
bellii	2.00	0.14	1–3	20	2300	33°20'; 70°19'
bisignatus	2.33	0.33	2–3	3	710	26°08'; 70°35'
chiliensis	2.40	0.51	1–4	5	800	33°28'; 70°32'
constanzae	2.57	0.14	2–3	14	2250	23°46'; 68°14'
dorbigni	5.00		5	1	4250	22°36'; 68°03'
eleodori	5.16	0.40	4–7	6	3670	27°04'; 69°10'
fabiani	3.25	0.25	3–4	4	2450	23°23'; 68°21'
fitzgeraldi	2.40	0.16	2–3	10	2900	32°50'; 70°08'
fuscus	2.00	0.00	2	4	950	33°35'; 70°28'
hellmichi	2.66	0.33	2–3	3	100	23°32'; 70°21'
jamesi	5.73	0.12	5–6	15	4350	18°10'; 69°25'
lemniscatus	2.25	0.16	1–4	20	950	33°35'; 70°28'
monticola	2.11	0.08	2–3	18	1850	33°46', 70°15'
nigroroseus	2.14	0.14	2–3	7	2300	23°02'; 68°04'
nigroviridis	3.10	0.39	2–5	8	2300	33°20'; 70°19'
nitidus	1.62	0.18	1–2	8	950	33°35'; 70°28'
ornatus	6.64	0.38	5–10	14	3710	19°15'; 68°43'
platei	2.25	0.25	2–3	4	200	27°03'; 70°51'
tenuis	2.57	0.11	2–3	19	600	34°03'; 70°35'

sealed and kept at −18°C until use. After extraction of the secretion, lizards were released at their sites of capture. The chemical characterization of the secretion of each species was performed after mixing secretions from three randomly selected individuals.

Solvent Purification. The *n*-hexane (Merck, chromatography grade) used for dissolving the precloacal secretions was previously purified by stirring over H_2SO_4 overnight, decanting, and stirring over K_2CO_3 for two hours. The solution was filtered, dried ($MgSO_4$), and redistilled before use. The glass ampoules used to keep secretions were heated to 150°C for four days before use.

GC-MS Analysis of Secretion Samples. The chemical characterization of the secretion of each species started with a pool of 150 μg of secretion, 50 μg from each individual (except in the case of *L. dorbigni*, where 50 μg were used from the single individual available). An aliquot (ca. 20 μl) of the mixture dissolved in *n*-hexane, containing 50 μg of the mixed secretion was submitted to the derivatization procedure (see below). The chemical characterization of the secretion of each individual

of *L. bellii* was performed by submitting directly 50 μg of each individual's secretion dissolved in *n*-hexane (ca. 20 μl), to the derivatization procedure.

Derivatization was performed by treating 50 μg of the secretion in v-vials with excess (5 μl) of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), and heating the closed vial to 90°C for 60 min. Thereafter, vials were cooled on ice, and excess MSTFA eliminated under a stream of dry nitrogen. The solution was concentrated to 2 μl and injected in the GC-MS.

Analyses were performed in a capillary column (Ultra 2, 25m $\times$ 0.2 mm ID) directly coupled to a mass detector with an integrated data system (GC model HP-5890, MD model HP-5972). Ionization by electron impact (70 eV) was carried out at 280°C. The GC oven was programmed to remain at 50°C for 10 min, then to increase up to 280°C at a rate of 5°C/min, and to remain at 280° for 45 min. The presence or absence of a given compound in the chromatographic profile of each *Liolaemus* species or individual of *L. bellii*, was determined by coinjection of commercial standards and comparison of retention times and mass spectra. GC peaks of coinjected compounds were considered coincident if retention times did not differ by more than $\pm$ 0.03 min. When standards were not available, spectra were compared with a library database by a reverse search technique, which verifies that main peaks in the reference spectrum are present in the unknown spectrum (Pesyna et al., 1976). Spectra were considered coincident if the similarity index was higher than 95% (Pesyna et al., 1976). Alternatively, mass fragmentation patterns were used to determine tentative chemical structures.

Data Analysis. Pearson product-moment correlations (Zar, 1984) were used to test relationships between normalized chromatographic areas of the different compounds in different species and environmental variables (altitude and latitude of capture site), and lizard characteristics (mean number of precloacal pores, snout-vent length, and weight of individuals, and mean weight of precloacal pore secretions). Analysis of similitude of the chemical composition of the secretions using normalized chromatographic areas, were performed among species and among individuals of *L. bellii*. Clusters of species and individuals of *L. bellii* were estimated by using unweighted pair-group of arithmetic average (UPGMA) as a linkage rule (Manly, 1994). The use of the comparative method has been recommended for interspecific comparisons (Martins and Hansen, 1996). However, since the phylogeny hypothesis proposed by Shultte et al. (2000) included only 11 of the 20 *Liolaemus* species in the present study, and those 11 species did not encompass a wide range of environments, a phylogenetic analysis was not performed.

RESULTS

GC-MS Analysis. The mean total mass of the secretions was 1.02 mg (SE = 0.25; N = 58, corresponding to the secretions that were analyzed by

GC-MS). The part soluble in *n*-hexane constituted approximately 67.5% (SE = 2.6; $N = 20$) of the total mass of the secretion. Eighteen compounds were found in the nine individuals of *L. bellii* studied (Table 2). Three different groups of compounds were found: *n*-alkanes, representing 5.8% (SE = 1.0; $N = 9$); long chain carboxylic acids, representing 48.7% (SE = 5.5; $N = 9$), and steroids, representing 45.5% (SE = 5.9; $N = 9$). Tricosane, tetradecanoic acid, hexadecanoic acid, hexadecenoic acid, octadecanoic acid, *Z*-9-octadecenoic acid, eicosanoic acid, and cholesterol, were present in all individuals analyzed. Among the major compounds found in the secretions of *L. bellii* are hexadecanoic and *Z*-9-octadecenoic acids, and cholesterol. Compounds found in *L. bellii* were also present in the other species. From the 20 *Liolaemus* species studied, a total of 49 compounds were identified (Table 3), which belong to the same three categories described for *L. bellii*: *n*-alkanes, representing 18.6% (SE = 4.0; $N = 20$);

TABLE 2. CHEMICAL COMPOSITION OF THE PRECLOACAL SECRETIONS OF NINE INDIVIDUALS OF *Liolaemus bellii*

			Normalized chomatographic areas (%)[a]								
No.	Compound	Im[b]	1	2	3	4	5	6	7	8	9
	n-Alkanes										
1	tricosane	A	1.1	1.5	3.3	1.5	3.5	1.5	1.5	1.1	1.0
2	tetracosane	A	nd	6.0	2.5	3.7	nd	nd	3.7	6.4	4.3
3	pentacosane	A	1.7	5.7	1.4	0.7	nd	0.7	0.7	nd	1.6
	Carboxylic acids										
4	2-hydroxy-propanoic	B	5.1	2.3	0.6	1.1	2.7	2.9	nd	4.8	3.8
5	hexanoic	B	3.6	nd	nd	nd	nd	2.0	nd	3.4	0.4
6	dodecanoic	B	0.8	0.5	0.5	0.5	1.1	0.5	nd	0.8	0.2
7	tetradecanoic	B	2.5	1.3	1.1	1.8	2.0	1.4	1.8	2.4	2.1
8	hexadecanoic	A	11.8	13.7	14.0	8.6	15.8	6.7	8.6	11.3	11.1
9	hexadecenoic	C	3.2	5.4	2.0	1.0	2.3	1.8	1.0	3.0	2.7
10	heptadecanoic	B	0.8	2.6	2.0	nd	1.1	nd	nd	0.8	0.6
11	octadecanoic	A	5.1	7.5	6.2	2.8	10.3	2.9	2.8	4.9	4.5
12	*Z*-9-octadecenoic	A	5.7	24.7	36.9	15.1	36.4	15.4	15.1	5.4	7.4
13	eicosanoic	B	3.0	5.7	5.4	3.6	6.0	3.7	3.6	2.9	2.3
14	docosanoic	A	4.6	4.8	1.4	nd	1.6	nd	nd	4.4	3.3
15	butanedioic	B	1.4	nd	nd	nd	nd	nd	nd	1.4	0.3
	Steroids										
16	cholesterol	A	6.7	12.5	18.2	55.9	8.6	56.8	56.0	38.1	44.5
17	cholestan-3-ol	C	40.0	5.9	nd	3.7	8.6	3.7	3.6	6.3	6.2
18	cholest-4-en-3-ol	C	2.9	nd	4.5	nd	nd	nd	1.6	2.6	3.7

[a] Normalized chromatographic areas expressed as percent of total detected compounds. nd = compound not detected.

[b] Identification method (Im) codes: A = comparison with authentic compounds; B = GC-MS comparison between the recorded and library mass spectra with similarity index higher than 95%; C = fragmentation patterns in the mass spectra.

TABLE 3. CHEMICAL COMPOSITION OF PRECLOACAL SECRETIONS OF TWENTY *Liolaemus* SPECIES

No.	Compound	Normalized chromatographic areas (%)[a,b]																			
		Im[c]	alt	bel	bis	chi	con	dor	ele	fab	fit	fus	hel	jam	lem	mon	nig	nir	nit	orn	pla	ten
	n- Alkanes																					
1	decane	A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.0	nd	nd	nd	nd	nd	nd
2	undecane	A	nd	nd	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	nd	0.3	1.1	nd	0.4	nd	nd	nd
3	dodecane	A	nd	nd	nd	nd	nd	nd	0.2	nd	nd	34.1	nd	nd	nd	0.5	2.5	1.2	nd	0.3	nd	1.4
4	tridecane	A	0.2	nd	nd	nd	0.5	0.3	0.7	nd	nd	nd	1.7	nd	4.1	1.6	3.3	1.0	nd	1.2	nd	1.1
5	tetradecane	A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.1	nd	nd	2.1	nd	nd	nd	2.6	nd	nd
6	pentadecane	A	nd	nd	0.3	0.1	0.8	nd	nd	nd	nd	nd	2.4	nd	1.2	1.7	1.8	0.8	0.6	1.5	3.0	1.2
7	hexadecane	A	nd	nd	nd	nd	1.1	nd	2.2	nd	nd	nd	4.1	nd	2.1	nd	3.4	1.4	nd	2.9	nd	1.4
8	heptadecane	A	nd	nd	nd	nd	2.3	2.4	4.8	nd	11.6	5.1	8.8	nd	3.2	5.9	6.4	2.4	nd	nd	nd	2.1
9	octadecane	A	nd	nd	nd	nd	nd	nd	6.5	nd	nd	nd	14.8	3.1	4.6	10.7	9.3	3.1	nd	nd	nd	1.6
10	nonadecane	A	nd	nd	nd	nd	nd	nd	3.5	nd	nd	nd	7.9	1.4	2.1	5.8	6.1	nd	nd	5.6	nd	nd
11	eicosane	A	nd	nd	1.7	nd	nd	nd	2.7	nd	nd	nd	7.5	nd	2.3	nd	nd	1.8	nd	6.4	0.5	0.7
12	heneicosane	A	nd	nd	0.8	nd	2.1	0.8	1.6	nd	nd	nd	4.8	0.9	nd	3.0	nd	nd	nd	4.1	1 nd	nd
13	docosane	A	nd	nd	nd	nd	2.9	1.2	1.3	nd	nd	nd	nd	1.5	18.9	nd	2.9	1.9	nd	5.1	nd	nd
14	tricosane	A	nd	1.1	0.8	1.3	2.1	0.7	1.2	nd	4.1	1.8	nd	1.5	1.6	nd	2.1	1.3	nd	3.5	1.9	2.7
15	tetracosane	A	nd	0.9	nd	nd	1.7	nd	0.7	nd	nd	nd	2.8	0.9	nd	nd	nd	nd	nd	1.5	nd	nd
16	pentacosane	A	nd	1.7	0.7	nd	1.9	nd	1.2	nd	nd	1.8	nd	nd	4.2	nd	0.5	1.2	nd	1.9	nd	nd
	Carboxylic acids																					
17	2-hydroxy-propanoic	B	nd	5.1	nd	nd	nd	4.6	9.2	7.7	5.0	nd	nd	nd	6.3	0.2	nd	6.7	0.2	0.5	12.8	2.5
18	hexanoic	B	nd	3.6	nd	nd	nd	0.4	nd	0.4	5.1	nd	nd	nd	nd	nd	nd	0.4	nd	0.2	nd	0.8
19	octanoic	B	0.3	nd	nd	nd	0.4	nd	nd	0.4	nd	nd	nd	nd	nd	nd	0.7	0.5	nd	nd	nd	1.0
20	nonanoic	B	0.2	nd	nd	nd	0.3	nd	nd	0.7	nd	nd	nd	nd	nd	nd	nd	0.4	nd	nd	nd	0.7

21	decanoic	B	0.2	nd	nd	nd	0.3	nd	nd	1.1	nd	nd	nd	nd	0.3	nd	nd	nd	nd	nd	nd	1.1
22	dodecanoic	B	1.0	0.8	nd	nd	2.6	0.8	1.0	nd	nd	nd	3.2	1.6	1.9	8.1	4.5	2.4	nd	5.8	nd	2.6
23	tetradecanoic	B	4.5	2.5	0.5	1.7	8.2	4.3	3.5	3.4	8.8	3.9	6.6	9.0	4.0	4.3	11.9	7.5	3.8	8.6	3.3	4.9
24	tetradecenoic	C	0.3	nd	nd	nd	1.5	nd	0.4	0.5	nd	nd	nd	nd	nd	nd	nd	0.9	nd	nd	nd	nd
25	tetradecanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.1	nd	nd	nd
26	hexadecanoic	A	41.9	11.9	13	32.6	29.2	14.3	2.4	9.9	32.1	23.7	5.3	1.8	10.6	3.8	19.0	24.5	14.0	17.2	26.0	21.1
27	hexadecanoic, methyl ester	B	nd	nd	0.2	nd	nd	nd	3.8	5.3	nd	nd	nd	1.4	1.4	nd	nd	8.5	15.9	nd	19.2	nd
28	hexadecenoic	C	16.6	3.2	7.6	4.9	9.1	4.1	3.0	3.9	5.8	0.2	1.1	4.7	3.7	4.5	7.9	8.1	3.6	11.5	0.9	5.8
29	heptadecanoic	B	0.9	0.8	nd	1.0	1.7	0.7	0.5	nd	1.6	nd	0.6	0.1	0.6	1.0	nd	1.4	nd	2.8	nd	0.8
30	heptadecenoic	C	1.5	nd	nd	nd	nd	nd	0.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
31	octadecanoic	A	7.8	5.1	0.8	9.0	8.6	4.3	4.8	3.5	12.5	10.3	2.8	0.7	1.7	6.2	2.4	5.5	6.2	4.3	10.9	6.0
32	octadecanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	1.2	2.5	nd	nd	nd	nd	4.3	nd	nd	3.1	10.3	nd	3.6	nd
33	z-9-octadecenoic	A	12.5	5.7	2.1	7.5	12.6	5.6	5.0	5.4	12.8	2.9	1.7	0.2	2.9	11.9	3.9	12.2	6.9	9.4	4.5	17.8
34	eicosanoic	B	0.4	3.0	6.4	12.1	2.8	3.3	4.5	11.3	nd	10.3	1.8	0.7	5.5	3.3	2.6	nd	2.3	nd	nd	4.9
35	eicosanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	1.4	6.4	nd	nd	nd	nd	nd	nd	nd	0.4	8.7	nd	2.2	nd
36	docosanoic	A	10.5	4.6	4.7	4.8	1.9	nd	6.0	nd	nd	4.8	1.3	0.7	3.4	4.3	0.7	nd	nd	nd	nd	5.2
37	docosanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	nd	9.0	nd	nd	nd	nd	nd	nd	nd	nd	6.2	nd	nd	nd
38	tetracosanoic	A	nd	nd	nd	2.8	nd	nd	nd	nd	nd	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd
39	tetracosanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.9	nd	nd	nd
40	hexacosanoic	B	nd	nd	nd	0.7	nd	nd	1.4	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	nd	nd	nd
41	hexacosanoic, methyl ester	B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.6	nd	nd	nd
42	butanedioic	B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	07	nd	nd
43	octadecadienoic, methyl ester	C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.2	nd	nd	nd	nd	nd	nd	nd

(*Continued*)

TABLE 3. CONTINUED

No.	Compound	Im[c]	Normalized chromatographic areas (%)[a,b] alt	bel	bis	chi	con	dor	ele	fab	fit	fus	hel	jam	lem	mon	nig	nir	nit	orn	pla	ten
	Steroids																					
44	cholesterol	A	0.9	6.7	52.98	2.6	4.1	50.6	24.3	28.7	0.7	1.1	17.5	9.4	3.8	20.0	6.7	1.3	15.4	1.7	11.0	0.8
45	cholest-4-en-3-one	C	0.2	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	59.8	nd	nd	nd	nd	nd	0.7	nd	nd
46	β-sitosterol	A	0.2	nd	nd	nd	1.4	1.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
47	cholestan-3-ol	C	nd	40.2	nd	13.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
48	3,5-dihydroxy-cholestane	C	nd	nd	nd	5.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
49	cholest-4-en-3-ol	C	nd	2.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.8

[a] Normalized chromatographic areas expressed as percent of total detected compounds; mass spectra with similarity index higher than 95%; C = fragmentation patterns in the mass spectra.

[b] *Liolaemus* species: alt = *L. alticolor*; bel = *L. bellii*; bis = *L. bisignatus*; chi = *L. chiliensis*; con = *L. constanzae*; dor = *L. dorbigni*; ele = *L. eleodori*; fab = *L. fabiani*; fit = *L. fitzgeraldi*; fus = *L. fuscus*; hel = *L. hellmichi*; jam = *L. jamesi*; lem = *L. lemniscatus*; mon = *L. monticola*; nig = *L. nigroviridis*; nir = *L. nigroroseus*; nit = *L. nitidus*; orn = *L. ornatus*; pla = *L. platei* and ten = *L. tenuis*.

[c] Identification methods (Im): A = comparison with authentic compounds; B = GC-MS comparison between recorded and library. C = Fragmentation patterns in the mass spectra (as trimethylsilyl derivatives if not otherwise stated): tetradecenoic acid, ms: m/z 55 (60), 73 (100), 81 (17), 117 (55), 145 (21), 166 (9), 208 (5), 283 (35), 298 (M^+, 4); hexadecenoic acid, ms: m/z 55 (50), 73 (100), 117 (63), 129 (49), 185 (6), 194 (10), 236 (8), 267 (2), 311 (53), 326 (M^+,8); heptadecenoic acid, ms: m/z 55 (40), 75 (100), 84 (14), 96 (20), 117 (60), 129 (31), 145 (17), 221 (7), 250 (4), 281 (10), 325 (25), 340 (M+, 3); octadecadienoic acid methyl ester, ms: m/z 55 (64), 57 (100), 81 (83), 95 (56), 109 (30), 121 (22), 135 (19), 150 (21), 164 (10), 220 (10), 262 (8), 294 (M^+,33); cholest-4-en-3-one, ms: m/z 55 (52), 69 (37), 95 (39), 124 (100), 147 (28), 229 (39), 271 (22), 298 (23), 342 (16), 384 (M+, 47); cholestan-3-ol, ms: m/z 55 (67), 81 (58), 95 (60), 121 (30), 149 (28), 165 (63), 194 (8), 215 (70), 233 (80), 248 (23), 262 (15), 331 (5), 355 (22), 373 (27), 388 (M^+, 100); dihydroxy-cholestane, ms: m/z 73 (69), 95 (50), 107 (37), 121 (31), 129 (100), 145 (28), 213 (14), 247 (14), 255 (11), 329 (65), 353 (28), 368 (42), 458 (M^+,19); cholest-4-en-3-ol, ms: m/z 55 (67), 78 (65), 95 (53), 107 (60), 119 (40), 133 (18), 147 (42), 161 (25), 173 (13), 213 (35), 229 (30), 255 (69), 273 (19), 353 (7), 371 (28), 386 (M^+,100).

long chain carboxylic acids, representing 61.5% (SE = 4.8; $N = 20$), and steroids, representing 19.9% (SE = 4.6; $N = 20$). All the species shared six compounds: cholesterol and five carboxylic acids (tetradecanoic, hexadecanoic, hexadecenoic, octadecanoic, and Z-9-octadecenoic). Hexadecanoic acid was the main compound of the carboxylic acid fraction, and cholesterol was the major steroidal compound.

Environmental Relationships. The number of precloacal pores (np) negatively correlated with the latitude ($r = -0.61$; $P = 0.004$) and positively correlated with the altitude ($r = 0.67$; $P = 0.001$) of the collection site. Different correlations were attempted among the normalized chromatographic areas of the different compounds and environmental or specific lizard characteristics. Nevertheless, the only significant correlations found were between the normalized chromatographic area of hexadecenoic acid and altitude ($r = 0.49$; $P = 0.028$), and latitude ($r = -0.44$; $P = 0.050$) of the collection site. Furthermore, for each species, the volatility of the secretion was estimated by using a melting point index (MPI) defined as the sum of the products between the normalized chromatographic areas and the melting point of each compound present in the secretion. No significant correlations were found between MPI and the environmental variables tested.

Similitude Analysis. The similitude analysis (UPGMA) performed with nine individuals of *L. bellii* (Figure 1A) shows the existence of variation in chemical composition at the individual level. A similitude analysis was performed with all 20 species together (Figure 1B). The relationship between the species could not be related to their phylogeny (Schulte et al., 2000).

DISCUSSION

Pheromone research in squamates is a relatively recent endeavor. The most precise knowledge of pheromonal compounds come from studies in snakes, where the nature of the active compounds has been elucidated in some cases (Mason, 1992, 1999; Mason et al., 1989). For lizards, most studies have dealt with the description of components that are part of secretions that are claimed to have pheromonal properties, rather than with the nature of the active compounds (Chauhan, 1986b; Alberts, 1990; Mason and Gutzke, 1990; Weldon et al., 1990; Alberts et al., 1992).

In all the *Liolaemus* species studied herein, the families of chemical components in the lipidic fractions of the precloacal secretions are similar to those reported for other lizards species, i.e., *n*-alkanes, carboxylic acid, and steroids (Chauhan, 1986b; Mason and Gutzke, 1990; Alberts et al., 1992). In *L. bellii*, the similitude analysis (Figure 1A) indicates that there are no two individuals with secretions having the same chemical composition. These differences among individuals may be important for self-recognition.

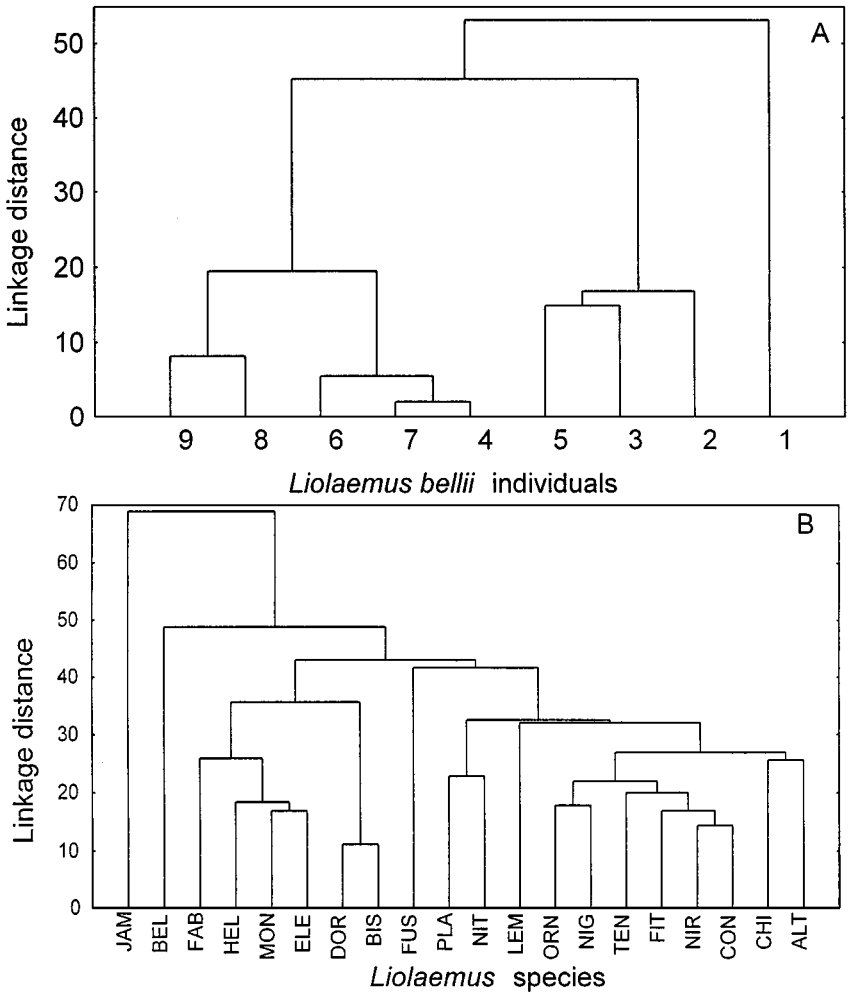

FIG. 1. Similitude analysis (UPGMA) of the normalized chromatographic areas of the different compounds found in the secretions of nine individuals of *Liolaemus bellii* (A) and in the secretions of 20 *Liolaemus species* (B). See Table 3 for meaning of abbreviations in Figure 1B.

The secretions of precloacal pores in *Liolaemus* contained a series of *n*-alkanes. In earlier studies with snake skin, these compounds were associated with sample contamination (Ahern and Downing, 1974), although it is now accepted that *n*-alkanes are components of saurian skin secretions (Weldon and Bagnall, 1987; Mason, 1992). Most of the *n*-alkanes of *Liolaemus* had an odd

number of carbon atoms. Since hydrocarbons of biogenetic origin generally consist of a greater proportion of odd over even number of carbon atoms (Weldon et al., 1990), *n*-alkanes in our samples are likely to be of biogenetic origin rather than a product of contamination. Nevertheless, the exact function of *n*-alkanes in *Liolaemus*, as well as in other lizard species (Mason and Gutzke, 1990), remains unknown.

Long chain carboxylic acids are common constituents of lizards skin and femoral glands (Mason and Gutzke, 1990; Alberts et al., 1992), and now they are reported in precloacal secretions of *Liolaemus* lizards. Carboxylic acids in *Liolaemus* belong to two categories: those common to all species analyzed (i.e., tetradecanoic, hexadecanoic, hexadecenoic, octadecanoic, and Z-9-octadecenoic), and those that are randomly distributed and present in minor amounts across the species (heptadecanoic, eicosanoic, and docosanoic). As previously suggested for snakes (Burken et al., 1985), compounds shared by different species may have a common function in all of them. Therefore, the five ubiquitous carboxylic acids may provide information related to genus status of the lizards (i.e., *Liolaemus*), while the randomly distributed carboxylic acids may be involved in specific discrimination, or species-specific functions.

The ubiquitous and most abundant carboxylic acid in *Liolaemus* secretions was hexadecanoic acid, also found in femoral secretions of *Iguana iguana* in breeding and nonbreeding seasons (Alberts et al., 1992). However, *Liolaemus* secretions showed differences with respect to previous records in squamates. In fact, hexanoic and nonanoic acids are reported for first time in squamate secretions. In addition, an important difference of *Liolaemus* secretions is the presence of compounds of lower molecular mass than compounds in other squamate species. Thus, in the leopard gecko, *n*-alkanes ranged from C_{18} to C_{32} and carboxylic acids from *n*-C_{16} to *n*-C_{18} (Mason, 1990); in *Iguana iguana*, carboxylic acids ranged from *n*-C_{14} to *n*-C_{26} (Alberts, 1992) and in the Florida indigo snake, carboxylic acids ranged from *n*-C_{12} to *n*-C_{18} (Ahern and Downing, 1974). In contrast, *Liolaemus* *n*-alkanes ranged from C_{10} to C_{25} and carboxylic acids from *n*-C_3 to *n*-C_{26}. Additional comparative research will be necessary to explain the biological implications of the presence of more volatile compounds in *Liolaemus* than in other squamates.

In squamates, precloacal (Chauhan, 1986a; Antoniazzi et al., 1993; Jared et al., 1999) and femoral glands (Weldon et al., 1990) have been described as exocrine organs producing a holocrine secretion (Alberts, 1990), which is constituted, among others, by long chain carboxylic acids (myristic, palmitic, stearic, and oleic acids), compounds normally present in internal tissues (Nicolaides, 1974). The presence of these carboxylic acids in the precloacal secretions of *Liolaemus* suggests the holocrine nature of the gland.

Cholesterol is of frequent occurrence in secretions involved in chemical communication, having been found in lizard skin (Weldon and Bagnall, 1987; Mason and Gutzke, 1990), femoral glands (Alberts et al., 1992) and now in precloacal pores in *Liolaemus* species. It has been suggested that steroids identified in lizards,

although not being sex steroids, may still give information about the sex of the individual (Mason and Gutzke, 1990). Since *Liolaemus* precloacal secretions analyzed were only from males, it may be speculated that the function of cholesterol derivatives is to provide information about the condition of the male, for example its dominance status or genetic quality (e.g., Martin and López, 2000).

It may be hypothesized that lizards deposit on the substrate a given "effective" amount of pheromones, which allows for efficient communication. However, these "effective" amounts may depend on the interplay of environmental or climatic conditions, and the volatility and chemical characteristics of the semiochemical. Thus, it would be expected that under conditions of high temperature, wind, and low atmospheric pressure, such as occur at higher altitudes and lower latitudes among the collecting sites studied, lizards would be equipped to produce higher quantities of secretion, or compounds in the secretions would be less volatile and chemically more stable. For *Liolaemus*, the main strategy seems to be the first one, given the significant relationships between number of precloacal pores with the altitude and latitude. Interestingly, the most volatile and one of the most chemically reactive members of the carboxylic acid family showed a negative correlation with latitude, and a positive one with altitude. However, the lack of correlation, between melting point index and both altitude and latitude, indicates that environmental conditions did not impose major changes in the pattern of chemical characteristics of the secretions.

Acknowledgments—This work was supported by the International Foundation for Science (IFS) through grant F 2933-1 awarded to AL and F 2934-1 awarded to CAE; FONDECYT through a postdoctoral fellowship awarded to AL; the Presidential Chair in Sciences awarded to HMN, and the International Program in the Chemical Sciences (IPICS). This work was part of the activities of the Center for Advanced Studies in Ecology and Research in Biodiversity funded by the Millennium Scientific Iniciative (P099-103F ICM). The authors thank the Servicio Agrícola y Ganadero (SAG), for lizard capture permit No. 992 (April, 1998) and No. 3112 (October 1999), and Corporación Nacional Forestal (CONAF) for permission No. 03 (December 1999), to capture lizards in protected areas.

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