ONTOGENETIC VARIATION OF PLASTRAL SPOTTING PATTERN IN *PHRYNOPS HILARII* (TESTUDINES, CHELIDAE)

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ABSTRACT

The plastral spotting variation in the chelid turtle *Phrynops hilarii* (Duméril & Bibron, 1835) in relation to sex, size, and geographic procedence of individuals was analyzed. States for qualitative characters were analyzed using non-parametric tests. Quantitative characters (shell and scute measurements) were standardized for body size by linear regression against carapace length, and were subjected to principal components analysis and canonical discriminant function analysis. Results suggest that increased plastral spotting is a polymorphic ontogenetic trait in *P. hilarii*. Neither hatchlings nor juveniles have plastral pattern moderately or heavily pigmented. The simplest pattern, however, may persist without changes in some adults. There are no differences between sexes. The spatial distribution of the plastral pattern is not ordered latitudinally or longitudinally, showing no relationship with gradients of elevation, temperature, or precipitation. This pattern trait lacks of taxonomic significance. The morphometric analysis failed to reveal any character of diagnostic utility in the plastron to support the possibility that these patterns correspond to different sympatric taxa.

KEYWORDS. Reptilia, Chelidae, *Phrynops*, pattern variation, South America.

INTRODUCTION

From its original description by Duméril & Bibron (1835), and through subsequent redescriptions (Boulenger, 1889; Freiberg, 1970; Pritchard, 1979; Ernst & Barbour, 1989; Cei, 1993), no variation in amount and distribution of pigment in the plastron of the turtle *Phrynops hilarii* has been recognized. Likewise, from its reinstatement to species rank by Freiberg (1970), *P. hilarii* is regarded as a homogeneous taxon, with no subspecies (Freiberg, 1977; Iverson, 1992; Cei, 1993). The revision of herpetological collections in Argentina, Brazil, and Uruguay revealed the existence in this species of variation in its plastral spotting, which might profitably be studied. The objectives are to determine the extent of this variation, its taxonomic significance (if any), and to analyze its geographic distribution and variation.

MATERIAL AND METHODS

Individuals of *P. hilarii* from throughout the species range were assembled from the following herpetological collections (acronyms of the institutions and name of curators in parentheses): Departamento Zoología Vertebrados, Universidad de la República, Montevideo (DZVU, F. Achával); Museo Nacional de Historia Natural, Montevideo (MHNM, H. Osorio and A. Mones); Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre (MCNZ, M. Leitão de Araujo); Museu de Zoologia, Universidade de São Paulo (MZSP, P. Vanzolini and A. Ramos Costa); Fundación Miguel Lillo, San Miguel de Tucumán (FML, S. Kretzschmar and G. Scrocchi); Museo Argentino de Ciencias Naturales, Buenos Aires (MACN, it includes specimens formerly at Centro Nacional de Investigaciones Iológicas, CENAI, G. Carrizo); Vivario del Museo de Ciencias Naturales e Históricas del Instituto Ruiz de Montoya, Posadas (IARM, A. Martínez); Museo de Ciencias Naturales y Antropológicas, Paraná, Entre Ríos (MER, C. Ceruti); Museo Provincial de Ciencias Naturales, Santa Fe (MFA, C. Virasoro and E. Fioramonti); Córdoba Zoo (ZOOC, D. Villarreal).

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Specimens examined. BRAZIL, **Rio Grande do Sul**: Santa Maria, Rio Vacacaí (MCNZ 3032); São Leopoldo (MCNZ 3641, 3642); Porto Alegre, Belém Novo (MCNZ 0797-0799, 2098), Delta do Jacuí, Ilha das Flores (MCNZ 6545), Ilha do Cipriano (MCNZ 7124), Ilha do Lage (MCNZ 8177, 8618, 8758), Ilha dos Marinheiros (MCNZ 8416); Viamão, Passo do Vigário (MCNZ 1115, 1173, 1174, 1204, 1225, 4959), Porto das Pombas (MCNZ 4958); Rio Guaíba (MCNZ 1254, 4957, 4961); Santo Antônio da Patrulha (MCNZ 6113); Tramandaí (MCNZ 4960); Palmares do Sul (MCNZ 6826); Cidreira (MCNZ 5091, 5092, 6546); Tapes, Lagoa Formosa (MCNZ 6320); São Lourenço do Sul (MCNZ 6092); Rio Grande, Taim (MCNZ 6261, 6262), Estação Ecológica do Taim (MCNZ 9114; MZSP 3056). ARGENTINA, **Santiago del Estero**: Alberdi, Campo Gallo (MFA 10, 11); **Chaco**: San Fernando, Barranqueras (MZSP 2677, 2679); **Misiones**: Candelaria, Estancia San Juan Pirahui, between road 12 and Loreto (MACN 35398); Provincia de Misiones (IARM w/Nº, four live specimens); **Corrientes**: Saladas (MACN 35396); Alrededores de Goya (FML 00028); **Santa Fe**: Villano, Chacoan zone (MACN 7011); 10 km S El Carril (MACN 30489); between San José del Rincón and Río Colastiné (MACN 34819); Santa Fe (MACN 7080; MFA 15); Rosario (MACN [ex CENAI 16]); **Córdoba**: Cruz del Eje, Embalse de Cruz del Eje (AC 265); Río Suquía (ZOOC w/Nº, alive); **Entre Ríos**: La Paz, 3 km N Piedras Blancas (MACN 30472); Paraná (MACN 30486; MER 178, 180); "Río Paraná" (MER 176, 191); Departamento Paraná (MER 189); Diamante (MER 194); Crespo (MACN 20547, 35397); Departamento Tala (MER 3861); Gualeguaychú, 5 km W Ceibas, Estancia La Peregrina (MACN 35399); **Buenos Aires**: Pilar, Zelaya (MACN 10805). URUGUAY, **Artigas**: Río Cuareim, Sepulturas, Picada del Negro Muerto (DZVU 863); Río Cuareim, 32 km NW Artigas, Estancia Yuquerí (DZVU 2315, 2343); Arroyo Cuaró, Paso Campamento, Estancia La Escondida (MHNM 01390); Arroyo Catalán Chico (DZVU 635, 2340); near to arroyo Catalán Chico, Estancia Chilo Martínez (DZVU 295); **Salto**: Río Arapey Grande, 4 km W Las Termas (DZVU 2341); **Paysandú**: Paysandú (MACN 34516, 34517); Río Uruguay, Paso de Vera, in front of Almirón (DZVU 2423); **Tacuarembó**: Río Negro, Paso de los Toros (MHNM 01386); Valle Edén (DZVU 728); **Cerro Largo**: Río Tacuarí, Picada de Medina (MHNM 01629); **Río Negro**: Near Estación Francia (MHNM 02568); Río Negro, 15 km SSE Nuevo Berlín (DZVU 742); Costa del río Negro, in front of Villa Soriano (DZVU 762-765, 775-777); Lagoon 4 km from río Negro, in front of Villa Soriano (DZVU 783-785); **Rocha**: Río Cebollatí, Picada de Techera (DZVU 370); **Soriano**: Arroyo del Perdido (DZVU 2309; MHNM 01388); Estancia Santa Elena (MHNM 01391).

The samples totalled 96 turtles of both sexes and unsexed juveniles, ranging from 31 mm to 380 mm carapace length, and included whole fluid-preserved or dry animals, single carapaces, and living specimens.

States for the following qualitative characters were recorded for each turtle. (1) Plastral pattern (PP states, fig. 1). Three states defined as: (state a) plastron surface yellowish, with dark marks (usually a symmetrical pattern of black spots) arranged in two concentric series roughly complete; (state b)

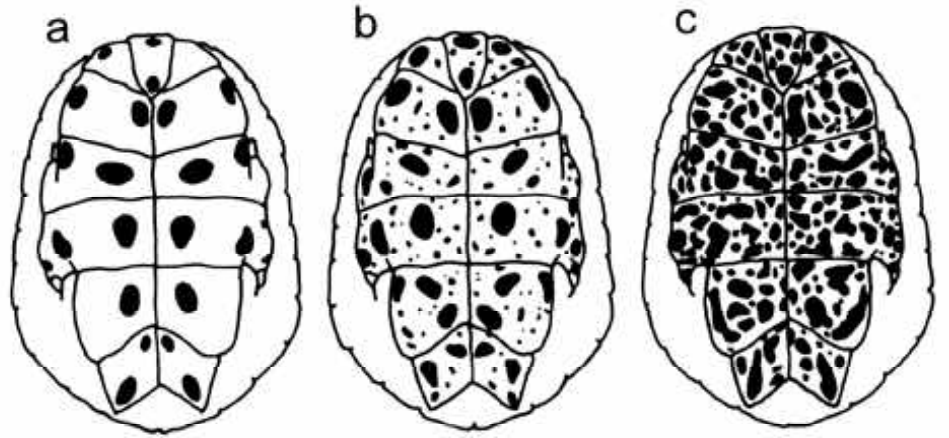


Fig. 1. Three states (a-c) of plastral pattern in *Phrynops hilarii* [a, plastron yellowish with dark marks arranged in two concentric series; b, marks as in (a) plus small dots irregularly dispersed; c, profuse irregular dark marks throughout the plastral surface].

marks as in (a), but with small black dots irregularly dispersed; (state c) profuse irregular black marks throughout the plastral surface, usually masking the symmetrical pattern; (2) edge of carapace yellowish from dorsal view; (3) number and relative size of spots in both the lower surface of marginals and bridge.

In order to analyze geographic variation, the range of natural distribution of the species (excluding the anthropochoric records cited by CABRERA, 1998) was divided into five areas (A-E, fig. 2) each of them related to a major hydrographic basin: A) Paraná and Paraguay Rivers basin; B) Uruguay River basin; C) river systems draining mainly to the east, tributaries of the Mirim and dos Patos lagoons; D) and E) endorheic basins in the Argentine political provinces of Santiago del Estero and Córdoba, respectively. Areas D and E (fig. 2) were later excluded from further analysis due to insufficient data availability. Qualitative characters were analyzed using non-parametric (Contingency Coefficient, and Kruskal-Wallis) tests (VANZOLINI, 1993).

Morphometric analysis were performed in order to analyze the potential relationship between

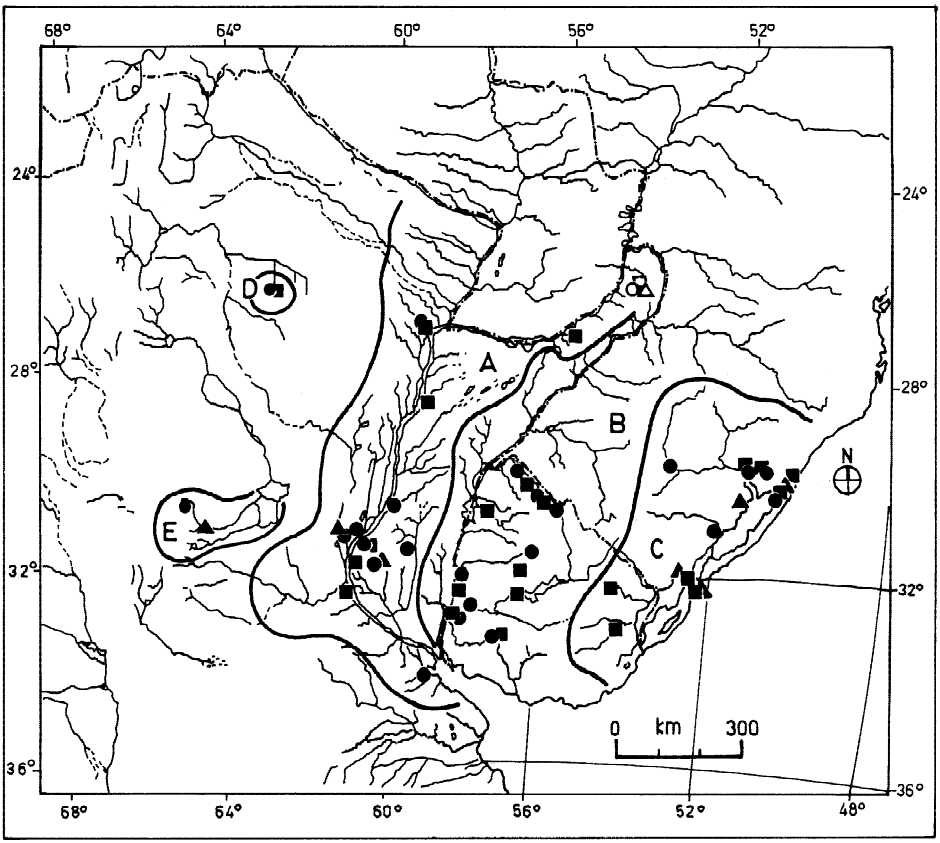


Fig. 2. Range of *Phrynops hilarii* showing the spatial distribution of plastral pattern states (l = a, n = b, s = c) and their relationship to hydrographic systems (A-E). A symbol may represent more than one specimen. Open symbols in Misiones indicate four individuals without exact procedence to indicate the co-occurrence of the three pattern states (a, plastron yellowish with dark marks arranged in two concentric series; b, marks as in (a) plus small dots irregularly dispersed; c, profuse irregular dark marks throughout the plastral surface; A, Paraná and Paraguay rivers basin; B, Uruguay river basin; C, river systems tributaries of the Mirim and dos Patos lagoons; D and E, endorheic basins in Santiago del Estero and Córdoba, respectively).

PP states and measurable shell features. Shell measurements (to the nearest mm) and scute measurements (to the nearest 0.5 mm) were taken on each specimen with carapace length ≥180 mm. From individuals smaller than this size only the carapace length and plastral pattern were recorded; because their shell outline and ratios differed markedly from those of adults, they were not included in the morphometric analyses.

Measurements, all straightline, included: maximum carapace length (CL); maximum plastron length (PL); midline length of plastron (MPL); midline length of the nuchal scute (NL); maximum length of the intergular scute (IL), and lengths of right interhumeral (IH), interpectoral (IP), interabdominal (IAb), interfemoral (IF), and interanal (IA) seams. Width measurements (straightline) included: maximum carapace width (CW); maximum width of first vertebral scute (V1); maximum width of fifth vertebral scute (V5); maximum width of plastral forelobe (PF); plastral forelobe width at the level of its base (PFB); width of the free edge of intergular scute (IW); maximum width of plastral hindlobe (PH); plastral hindlobe width at the level of its base (PHB).

These characters were standardized for body size by linear regression against CL, and the residuals were used in the statistical calculations. Arguments for the use of residuals as input variables were noted in CABRERA & COLANTONIO (1997) and references therein. The residuals were then subjected to ANOVA and MANOVA tests, principal components analysis (PCA) of the character correlation matrix, and canonical discriminant function analysis (DFA), using the SPSS (Statistical Package for Social Sciences) software version 5.0.1.

RESULTS AND DISCUSSION

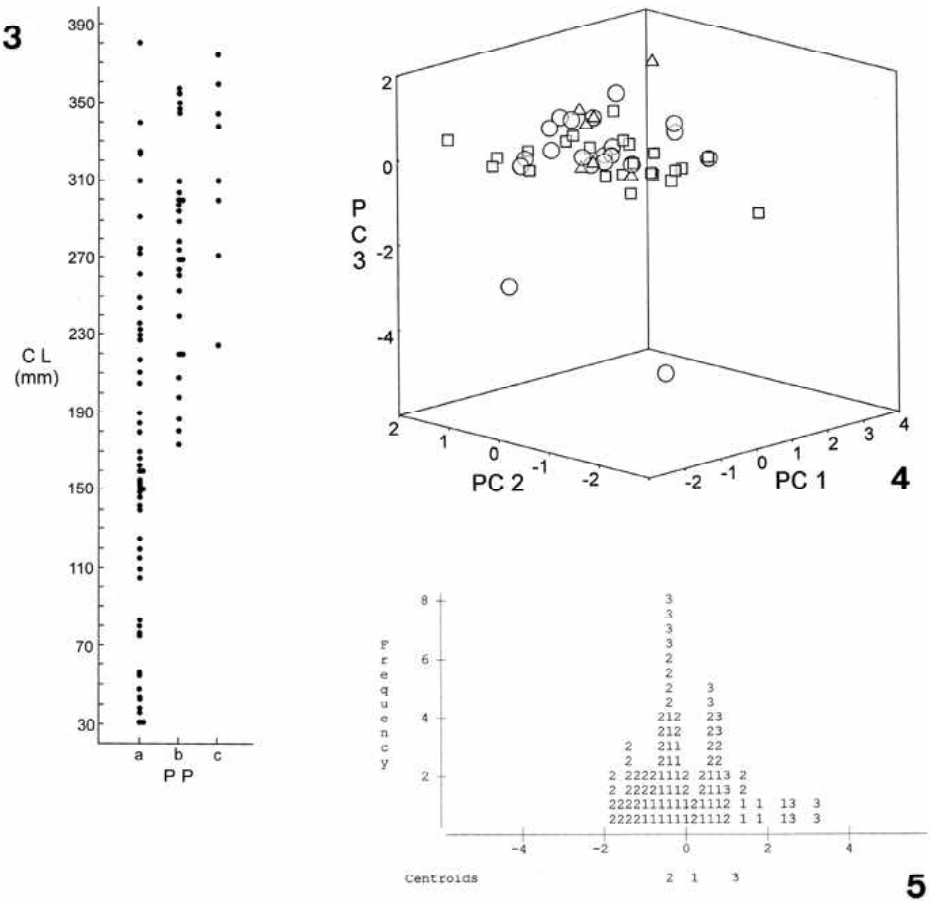
Qualitative characters analysis. The following characters were independent of geographic procedence in *Phrynops hilarii*: a yellow rim around the carapacial margin, although some more marked in juveniles, was always visible dorsally; number and relative size of dark spots on both the plastral aspect of the marginal scutes and the bridge always showed a direct relationship to the abundance and size of the plastral spots. Consequently, these characters were not analyzed further.

All individuals adscribed well to one of the three alternative states of plastral pattern (PP). Although intermediate stages are expectable as a hatchling with state a grows into an adult with state b or c, the transition is perhaps relatively rapid; we consequently used PP states as comparative categories. In adults the relationship between sex and PP state was not significant (Contingency Coefficient C = 0.192, $P = 0.316$), suggesting independence between sex and PP states (tab. I). When PP was analyzed in relation to size we found that hatchlings and juveniles with CL up to 170 mm showed only the PP state a (fig. 3). The PP state c was only present in large individuals, in the samples examined those over 270 mm CL (except for one specimen with CL = 225 mm). The relationship between CL as an index of overall body size (and for extension, relative age), and the PP states was significant (Kruskal-Wallis H = 33.85, df = 2, $P<0.001$), with larger animals having more spotting.

The relationship among geographic areas A-C and PP states was not significant (C = 0.227, $P = 0.357$) (tab. II). The variation in plastral pattern was not clinal. There are localities where any two patterns were present (a and c states in Crespo, Entre Ríos; a

Table I. Plastral pattern (PP) states and sexes of *Phrynops hilarii*. Number of cases; in parentheses, column percentages [a, plastron yellowish with dark marks arranged in two concentric series; b, marks as in (a) plus small dots irregularly dispersed; c, profuse irregular dark marks throughout the plastral surface].

PP states	Males	Females	Total
a	11 (28.2)	10 (47.6)	21 (35.0)
b	22 (56.4)	9 (42.9)	31 (51.7)
c	6 (15.4)	2 (9.5)	8 (13.3)
Total	39	21	60 (100.0)



Figs. 3-5. 3, Relationship between plastral pattern states (PP) and maximum carapace length (CL) in *Phrynops hilarii*. Each dot = one specimen; 4, plot of plastral pattern states (○ = a, □ = b, △ = c). Three first principal components (PC1-PC3) based on PC analysis of size-adjusted residuals of measurable characters. Each symbol = one specimen; 5, discriminant scores for the only canonical function for a, b, and c (represented respectively by 1, 2, and 3). Each number = one specimen (1, plastron yellowish with dark marks arranged in two concentric series; 2, marks as in (1) plus small dots irregularly dispersed; 3, profuse irregular dark marks throughout the plastral surface).

Table II. Contingency table for plastral pattern (PP) states of *Phrynops hilarii*, and geographic areas. Number of cases; in parentheses, column percentages (a, plastron yellowish with dark marks arranged in two concentric series; b, marks as in [a] plus small dots irregularly dispersed; c, profuse irregular dark marks throughout the plastral surface; A, Paraná and Paraguay rivers basin; B, Uruguay river basin; C, river systems tributaries of the Mirim and dos Patos lagoons).

States	Areas			Total
	A	B	C	
a	11(55.0)	15(53.6)	19 (57.6)	45 (55.6)
b	7(35.0)	13(46.4)	10 (30.3)	30 (37.0)
c	2(10.0)	0 (0.0)	4 (12.1)	6 (7.4)
Total	20	28	33	81(100.0)

Table III. Loadings for the most influential residuals from principal components (PC) analysis (IA, interanal seam length; IF, interfemoral seam length; IH, interhumeral seam length; MPL, midline length of plastron; NL, midline length of the nuchal scute; PF, maximum width of plastral forelobe; PFB, plastral forelobe width at its base; PH, maximum width of plastral hindlobe; PHB, plastral hindlobe width at its base; PL, maximum plastron length; V5, maximum width of fifth vertebral scute).

Residuals	PC1	PC2	PC3	PC4	PC5	PC6
PF	0.859	-0.189	0.014	0.051	0.273	0.012
PFB	0.928	-0.044	0.091	-0.033	0.142	0.101
PH	0.890	0.022	0.143	0.047	0.014	0.065
PHB	0.879	0.128	0.256	-0.023	-0.007	-0.042
V5	0.163	0.201	0.172	-0.103	-0.028	0.810
PL	0.126	0.083	0.800	-0.020	-0.045	0.098
NL	0.070	-0.144	-0.098	0.199	0.878	0.093
MPL	0.153	-0.149	0.864	0.031	-0.028	0.060
IA	0.408	-0.045	0.130	0.748	0.246	0.028
IF	0.161	0.083	0.079	-0.899	0.086	0.093
IH	0.319	0.723	0.060	0.143	0.179	0.164
Eigenvalue	5.18	2.30	1.68	1.40	1.13	1.08
Cum.Percent	30.50	44.0	53.9	62.1	68.7	75.1

and b states in Arroyo Catalán Chico, Artigas Department; b and c states in Estação Ecológica do Taim, Rio Grande do Sul). Moreover, geographic proximity and/or presence in the same major river basin of specimens showing any third state warrants the co-occurrence of the three states in many cases. The absence of the c state in area B is interpreted as a sampling artifact. The spatial distribution of the plastral patterns were not ordered latitudinally or longitudinally, showing no relationship with gradients of elevation, temperature, or precipitation.

Morphometric analysis. Residuals of four characters (IW, PHB, PL, and MPL) showed no univariate homogeneity of variance to Cochrans C and Bartlett-Box F tests, and were excluded from multivariate analysis of variance. Multivariate differences among PP states were not significant (Pillais F = 0.63105, df = 26, P = 0.235; Hotellings F = 1.04105, df = 26, P = 0.181; Wilks F = 0.45016, df = 26, P = 0.206). Only the character IAb showed significant differences among PP states (F = 7.456, P = 0.002).

The PCA results showed no clear separation among the PP states (fig. 4). Six principal components whose eigenvalues were higher than one were obtained (tab. III). The first principal component (PC1) accounted for 30.5% of the total variation. Characters more influential to PC1 were PF, PFB, PH, and PHB. The second principal component (PC2) accounted for 13.5% of the remaining variation. The most influential

Table IV. Summary of classification results of function discriminant analysis for the plastral pattern states a-c in *Phrynops hilarii*, based on size-adjusted residuals of measurable characters [a, plastron yellowish with dark marks arranged in two concentric series; b, marks as in (a) plus small dots irregularly dispersed; c, profuse irregular dark marks throughout the plastral surface].

Actual group	Nº of cases	Predicted group membership (%) a	b	c
a	20	8 (40.0)	11 (55.0)	1 (5.0)
b	23	8 (34.8)	15 (65.2)	0 (0.0)
c	7	3 (42.8)	2 (28.6)	2 (28.6)

character was IH. The third principal component (PC3) accounted for 9.9% of the remaining variation. Characters most influential loading on PC3 were PL and MPL. Fourth to sixth components (PC4 to PC6) accounted for low percentages of the remaining variation (tab. III).

The DFA was congruent with the PCA showing that a clear separation among PP states was not evident (fig. 5). The percentage of cases correctly classified in its actual group was only 50% (tab. IV). The only canonical discriminant function explained 100% of the variability among PP states (Eigenvalue = 0.32, df = 2, $P = 0.002$), and was represented by the IAb character.

The morphometric analysis failed to reveal any character, or combination of them, of some diagnostic utility to support the possibility that these patterns correspond to different sympatric taxa. The only character statistically significant to ANOVA was the length of interabdominal seam, in agreement with the discriminant function obtained. However, this character as well as other midseam scute lengths with which the plastral formula is calculated, is variable in this species (Ernst & Barbour, 1989) as well as in turtles in general (Lovich & Ernst, 1989). These latter authors showed for seven families of chelonians that plastral formulae may differ between sexes and among size classes in a given taxon. Therefore, the use of these proportions as key characters to characterize individual species must be cautiously applied.

Results suggest that increased plastral spotting in *P. hilarii* is a polymorphic ontogenetic trait. Neither hatchlings nor juveniles ever have plastral pattern moderately or heavily pigmented (the states b and c). However, the simplest pattern (the a state), that is found in all of the specimens under 17 cm carapace length, persists without changes in some adults up to, at least, 38 cm of carapace length.

Results also show that this trait lacks of taxonomic significance. Notwithstanding, the description of plastral coloration of *P. hilarii* as referred hitherto by most authors is incomplete, and should be expanded to the following. Plastron pale yellow with black or dark brown spots. The pattern in hatchlings and juveniles up to 17 cm of carapace length is formed by two concentric series of circular or eliptical blotches, roughly symmetrically arranged, on most or all of the scutes. This pattern may persist unchanged into adulthood, even in very large individuals, or may be replaced by one of these variants: (a) a pattern showing spotting similar to that previously described accompanied by dots more or less abundant randomly dispersed on the scutes, or (b) a pattern formed by dark irregular and abundant spots all over the plastral surface, without persistence of the juvenil symmetric pattern. On the inframarginal and bridge surfaces the pattern corresponds to that of the plastron itself.

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