

GEOGRAPHIC VARIATION IN ADVERTISEMENT CALL AND GENETIC STRUCTURE OF *COLOSTETHUS PALMATUS* (ANURA, DENDROBATIDAE) FROM THE COLOMBIAN ANDES

XIMENA E. BERNAL^{1,2,3}, CARLOS GUARNIZO¹, AND HORST LÜDDECKE¹

¹*Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, A.A. 4976, Colombia*

ABSTRACT: Among frogs endemic to the Colombian Andes, the dendrobatid *Colostethus palmatus* has an exceptionally wide geographic distribution. We examined the patterns of variation in calls and genetic properties to determine whether this variation is associated with geographic barriers and distances between populations. We analyzed variation in advertisement calls and RAPD products in seven populations along a 346-km transect from south to north throughout the Cordillera Oriental covering an extensive part of the geographical range of *C. palmatus*. Populations of this species inhabit both slopes of the Cordillera Oriental, and are separated by a continuous ridge along these mountains. We found more inter- than intrapopulation variation. Four call variables and male snout-vent length exhibited clinal variation, generating a predictable geographic pattern which coincided with a climatic gradient of increasing moisture and seasonality. The genetic relations between populations are explained by their location on the Andes, particularly by the side of the slope. Differences between populations in four call characteristics and genetic distance were significantly correlated with linear geographic distances between populations on the same slope. We found evidence for genetic and bioacoustic differentiation between populations living on opposite slopes.

Key words: Call variation; Gene-flow barrier; Isolation-by-distance; Mating signals; RAPD

GEOGRAPHIC variation in the advertisement call of frogs and toads is commonly observed among conspecific populations of widespread anuran species (reviewed in Gerhardt, 1994 and in Wilczynski and Ryan, 1999). Variation in this trait has received considerable attention due to the important role that this signal plays in mating behavior (Grohová et al., 1996; Hasegawa et al., 1999; Nevo and Capranica, 1985; Ryan et al., 1996). Studies of intraspecific variation of mating calls have proven valuable for understanding the evolution of mating signals (Boul and Ryan, 2004; Loftus-Hills and Littlejohn, 1992; Ryan et al., 1996; Sullivan et al., 2000), and clarifying taxonomic status (Castellano et al., 2000; Littlejohn, 1957; Roberts, 1997; Sullivan, 1989). Several models have been proposed to explain variation among conspecific populations. In species with large distributions relative to their dispersal abilities, isolation by distance takes place, and geographic variation takes the form of gradients or clines. The isolation-by-distance model was first proposed by Wright (1943) and predicts that populations geographically closer to each other should be separated by smaller pheno-

typic and genetic distances, since the homogenizing effects of gene flow are related to the geographic distance between populations. As geographic distance increases, however, differences between populations increase stochastically (Avice, 1994; Hutchison and Templeton, 1999; Nei, 1972; Neigel, 1997; Slatkin, 1993; Wöhrmann and Jain, 1990). In the absence of physical barriers to dispersion, gene flow has been viewed as a powerful cohesive force (Slatkin, 1987). Nevertheless, past historical events may generate dispersal barriers over the distributional range of a species. Barriers to dispersal such as mountains, rivers or deserts, lead to vicariant splitting of once continuously distributed species (Gascon, 1996; Grohová et al., 1996; Toda et al., 1998).

The dendrobatid *Colostethus palmatus* is endemic to the Colombian Andes. This frog inhabits parts of the eastern slopes of the Cordillera Central and both slopes of the Cordillera Oriental within an altitudinal range of 350–2200 m (Cochran and Goin, 1970; Rivero, 1988). This extensive distribution is remarkable for Andean frogs, although not unique (Grant and Castro, 1998). *Colostethus palmatus* has not been reported from altitudes above 2500 m (Ruiz-Carranza et al., 1996), suggesting that populations living on opposite slopes of the Cordillera Oriental are separated

² PRESENT ADDRESS: Section of Integrative Biology C0930, University of Texas, Austin, TX 78712, USA.

³ CORRESPONDENCE: e-mail, xbernal@mail.utexas.edu

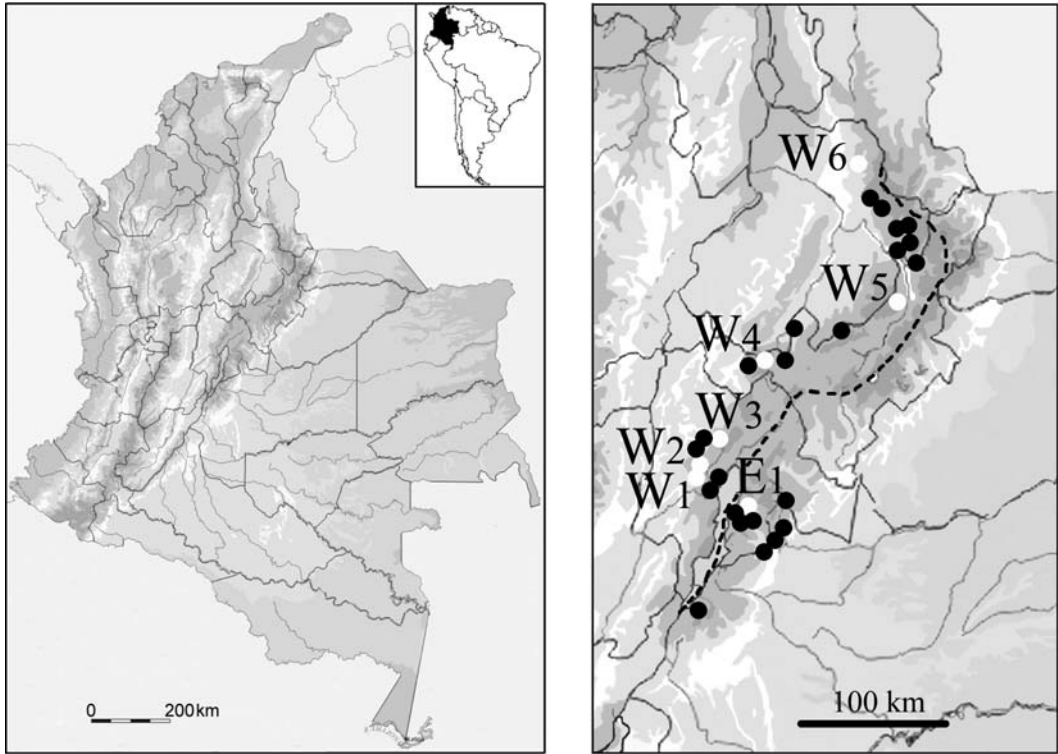


FIG. 1.—Map of study area in Colombia indicating the geographical position of *C. palmatus* populations sampled in the Cordillera Oriental. The sampled sites, white dots, are named accordingly to their position on the mountains, W = western slope, E = eastern slope. The black dots indicate sites in which *C. palmatus* has been reported in the Cordillera Oriental (based on the specimens found at the Instituto de Ciencias Naturales, Universidad Nacional, Colombia). The dotted line indicates the uninterrupted Cocuy ridge of at least 2500 m altitude.

by a ridge of at least 2500 m high (Cocuy ridge, Fig. 1; Rivero, 1988). This ridge reaches 2500 m elevation near Ocaña (Norte de Santander), north of the northernmost record of the presently known distribution of *C. palmatus*, which does not include the northern foothills (Ruiz-Carranza et al., 1996). The ridge extends at a straight-line distance of about 530 km and ends in the south in the depression of Paso de Andalucía in southern Meta, as low as 2000 m (Rivero, 1988). Today's distributional pattern probably originated from the final uplift of the Cordillera Oriental when the mountains reached their present elevation between 2–5 mya (Gregory-Wodzicki, 2000), separating populations on opposite slopes.

In the Andean slopes, most species of frogs have restricted ranges and species replace one another along the geographic axis of the Cordillera, while few species have extensive

distributions (Lynch and Duellman, 1997; Lynch et al., 1997). To our knowledge no studies have investigated the geographic variation of widespread Andean species to date. Given the broad geographical range of *C. palmatus* in the Cordillera Oriental across a potential barrier to gene flow, this species provides an excellent opportunity for studying intraspecific variation in vocalizations in conjunction with the genetic relations among such populations. We examined the geographic variation in the advertisement call in relation to the population dynamics in this mountain frog in the neotropics addressing three major questions: (1) Do advertisement calls and genetic structure show significant interpopulational differences? (2) Is call divergence and genetic distance between populations correlated with the geographic distance separating them? (3) Is call divergence and genetic

distance between populations associated with geographic barriers?

MATERIALS AND METHODS

Field Work

From April 1999–September 2000, we visited seven sites throughout the species' range in the Cordillera Oriental of Colombia (Fig. 1). One site (E_1) was located at 2000 m on the eastern slope while all other sites were between 1420 m and 2020 m elevation on the western slope (Fig. 1, Table 1). Multiple attempts to sample additional populations on the eastern slope, at latitudes different from E_1 , failed. We encountered several problems; on the eastern slope this species seems to be less abundant, the road system is deficient and there is a higher risk associated to doing field work in that area due to the political situation of Colombia. This situation is reflected in the fewer and more localized records from that side of the Cordillera (Fig. 1). At each of the six sites (except W_3) we tape recorded 10 consecutive advertisement calls from 10 males, following standard procedures (Heyer, 1994). Calls from W_3 could not be recorded because in this population males called from extremely secluded sites. Given the elusiveness of the frogs, attempts to capture the calling male were not always successful. In each population, we collected 7–15 males, which were not necessarily the focal males (Table 1). We measured snout–vent length (SVL) of captured individuals (± 0.05 mm), and in most populations we retained one male and a female per population as voucher specimens (Appendix I), while the others were released. Calls were recorded using a Sony WM D6C Professional Walkman tape recorder and an AKG 1200 directional microphone. Immediately after a recording, we measured the substrate temperature at the calling site of each focal male. Furthermore, at each of the five sites we removed tissue from six individuals, which was either a finger of an adult or a slice from the tail of a tadpole, and preserved it in individual Eppendorff capsules with 97% ethanol. Tissue from populations W_2 and W_5 could not be analyzed due to budget constraints.

Call Analysis

Calls were digitized at 22 kHz and analyzed on a Power Macintosh computer using CANARY (Charif et al., 1995). All calls were measured according to specifications given by Cocroft and Ryan (1995). The following six call characters were used to describe each call: dominant call frequency, number of notes/call, call duration, call rise time, note repetition rate in the call section where it was constant, and call repetition rate.

Genetic Analysis

DNA was extracted using the kits "High Pure PCR Template Preparation Kit", and "Wizard Genomic DNA Purification Kit" (Promega). The Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR reaction) was run using a "Ready-to-go" kit (Amersham Pharmacia Biotech). The mix containing the reaction bead (AmpliTaq™ DNA polymerase and Stoffel fragment), 0.4 mM of each dNTP, 2.5 μ g BSA and buffer (3mM $MgCl_2$, 30mM KCl and 10mM Tris, pH 8.3), all in a reaction volume of 19 ml distilled water and 5 ml primer, was partitioned into four tubes with 6 μ l each. For the RAPD-PCR reaction, we added 1 μ l of DNA to each tube. The PCR cycling procedure, carried out in a Mini Cycler (MJ Research) was as follows: after an initial denaturation step of 5 min at 95 C, 45 cycles of denaturation for 1 min at 95 C, primer annealing for 1 min at 36 C, and extension for 2 min at 72 C. We used four primers (5'-GGT GCG GGA A-3', 5'-AAG AGC CCG T-3', 5'-AAC GCG AAA C-3', 5'-CCC GTC AGC A-3'). PCR-products were separated on polyacrylamid gels in batches of ten individuals (two from each population), a control without DNA, and the RAPD product (made with the corresponding primer) from one of the individuals run in the first electrophoresis, in order to compare band migration between different gels. Bands were treated with silver nitrate (DNA Silver Staining Kit, Pharmacia Biotech), then digitized and aligned.

Analysis of Geographic Variation

Data were processed using SYSTAT (Wilkinson, 1991). Descriptive statistics were calculated for every call character in each

TABLE 1.—Means ± SE of all call characters measured at the study localities over the range of *C. palmatus*. There is one population on the eastern slope (E₁) and there are six populations on the western slope of the Cordillera Oriental, organized from south to north (W₁–W₆). We show the name of the closest town for each locality, the elevation (masl), and the latitude (LAT) and longitude (LONG) for each specific sampling site. Abbreviations: NOTR, note repetition rate (notes/s); DMFQ, dominant frequency (Hz); CDUR, call duration (s); PRIS, rise time expressed as a percentage of duration of call; RIST, rise time (s); NUNO, number of notes; CRAT, call repetition rate (calls/min); SVL, snout-vent length (mm). Ten individuals (10 calls/individual) were recorded in each population. The number of males measured is shown in parenthesis for each population.

	Locality		NOTR	DMFQ	CDUR	PRIS	RIST	NUNO	CRAT	SVL (<i>n</i>)
	Town	Altitude (LAT/LONG)								
E1	Ubaque	2000 m (04° 26'/73° 55')	7.57 ± 0.30	2579 ± 47.6	21.67 ± 1.56	65.69 ± 3.35	14.27 ± 1.21	165.07 ± 15.12	0.934 ± 0.06	28.0 ± 0.34 (15)
W1	Mesitas	1420 m (04° 33'/74° 25')	8.70 ± 0.27	2533 ± 26.3	27.51 ± 1.41	56.43 ± 4.23	15.52 ± 1.36	241.21 ± 16.69	1.026 ± 0.08	31.6 ± 1.0 (9)
W2	Tena	1980 m (04° 41'/74° 22')	8.117 ± 0.20	2437 ± 26.7	25.58 ± 1.09	58.77 ± 5.83	15.02 ± 1.73	208.40 ± 11.79	1.040 ± 0.04	31.7 ± 0.28 (13)
W3	San Francisco	1600 m (04° 50'/74° 15')	—	—	—	—	—	—	—	—
W4	Chiquinquirá	2020 m (05° 38'/73° 54')	8.25 ± 0.19	2409 ± 21.1	35.47 ± 1.26	68.57 ± 2.28	24.50 ± 1.30	293.12 ± 13.85	0.779 ± 0.04	31.9 ± 0.49 (7)
W5	Oiba	1530 m (06° 14'/73° 18')	8.45 ± 0.26	2212 ± 30.7	32.16 ± 1.33	60.31 ± 3.82	19.48 ± 1.23	273.33 ± 16.76	0.763 ± 0.08	32.8 ± 0.90 (9)
W6	Suratá	1900 m (07° 22'/73° 01')	7.125 ± 0.10	2083 ± 22.6	41.51 ± 3.19	63.75 ± 3.01	25.83 ± 1.82	296.85 ± 22.50	0.658 ± 0.08	32.0 ± 0.41 (10)
Effect of Temperature										
	<i>F</i> -ratio		4.806	3.300	0.351	0.882	0.979	0.202	0.083	—
	<i>P</i>		**	ns	ns	ns	ns	ns	ns	—
Among Populations										
	<i>F</i> -ratio		4.372	40.807	15.409	1.182	10.834	9.543	5.716	11.11
	<i>P</i>		***	***	***	ns	***	***	***	***

population. For interpopulational call comparisons we performed separate univariate analysis of covariance (ANCOVA) for each call character, using temperature as a covariate. We calculated Spearman rank correlations between populational averages of male SVL and averages of each call characteristic. We also independently correlate latitude of the study sites with SVL and each call trait to investigate clinal variation.

To establish how call characteristics varied among populations, we performed a discriminant function analysis. To evaluate associations between geographic distance/side of the ridge and call dissimilarity, we performed Mantel tests using MANTEL (Liedloff, 1999). Ten thousand iterations were conducted to evaluate the significance of the results. The Mantel test calculates correlations among similarity/dissimilarity and distance matrices considering that the same data point is used for multiple comparisons, and has proven to be an effective method for analysis in patterns of association between similarity/dissimilarity matrices (Castellano and Balleto, 2002). We used a dissimilarity matrix of Euclidean distances between means of all call variables, and correlated it with a matrix of the straight-line distances between all pairs of study sites along the western slope and across the ridge. A partial Mantel test was also performed using the program Fstat293 (Goudet, 2001).

For the genetic analysis, only clearly visible RAPD bands were taken into account to establish a binary matrix for all loci of all individuals, assigning 1 to the presence, and 0 to the absence of a band (Appendix II). The program PAUP* (Swofford, 2003) was used to calculate Nei genetic distances between all individuals and populations, and to calculate UPGMA and Neighbor Joining trees. Non parametric bootstrap (1000 replicates) was used to find support for the tree branches. We used *C. subpunctatus* from Bogotá (04° 38' N, 74° 06' W) as the outgroup to root the trees. This species belongs to a different group within the genus (Rivero and Serna, 1988). To evaluate the relationship between geographical distance among populations and genetic distance (Nei distance), as well as migration rate (Nm), Mantel tests were performed. Partial Mantel tests were used to examine the effect of the ridge on genetic distance and

migration rate. We also performed a discriminant function analysis using the binary matrix (30 loci per individual) to evaluate how genetic structure varies among populations in a comparable way with the analysis performed with the call characteristics.

RESULTS

General Description of the Advertisement Call

The advertisement call of *C. palmatus* had the same basic structure in all populations. It consisted of a long train of notes emitted in fast succession (Fig. 2A). Note repetition rate accelerated over the first third to half of the call. Each note was a single pulse (Fig. 2B), which concentrated call energy within a narrow frequency band (Fig. 2C), and sounded like a melodic whistle to the human ear.

Call Differences between Populations

Univariate analysis established that there was significant geographic variation in all five call characteristics measured, and in call repetition rate (Table 1, Fig. 3). No significant thermal effect on call characteristics was found across the temperature range encountered, except for note repetition rate (ANCOVA, $F_{1,53} = 7.730$, $P = 0.008$; for all other characteristics, $P > 0.095$). Populations with higher temperatures, like W_1 and W_5 , had higher note repetition rates. Across populations, only call dominant frequency was significantly correlated with male SVL (Spearman rank correlation coefficient, $r_s = -0.94$, $P = 0.035$, $n = 6$). No differences in call frequency remained after controlling for differences in SVL (using residuals of dominant frequency; Spearman rank correlation coefficient, $r_s = -0.60$, $P = 0.18$, $n = 6$). SVL of males was not correlated with the altitude of the study sites (Spearman rank correlation coefficient, $r_s = -0.20$, $P = 0.655$, $n = 6$), but was correlated with their latitude (Spearman rank correlation coefficient, SVL: $r_s = 0.94$, $P = 0.03$, $n = 6$). The main difference in SVL was between the eastern population and the western ones, but even when E_1 was removed from the analysis there still was a significant clinal change in body size from south to north (Spearman rank correlation coefficient, SVL: $r_s = 0.90$, $P = 0.04$, $n = 5$). Female SVL was

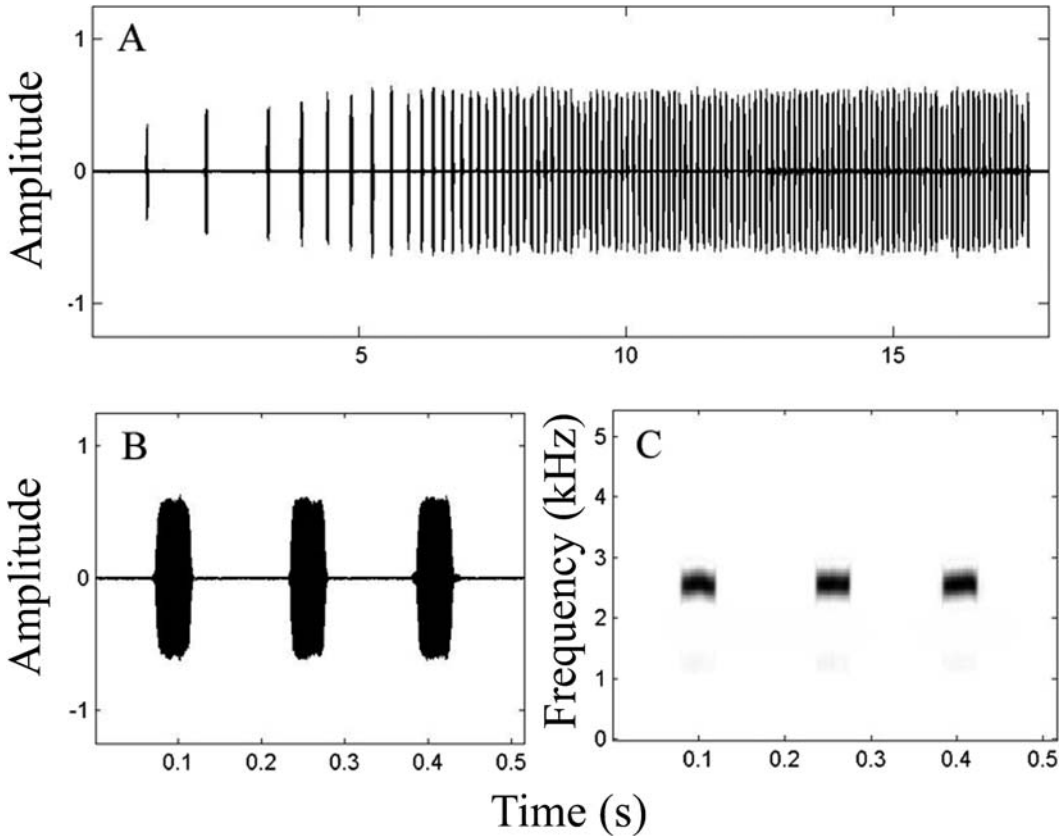


FIG. 2.—Representative advertisement call of *C. palmatus*. (A) Oscillogram of a complete call. (B) Oscillogram of three notes from the central part of the call. (C) Sonogram of three notes.

higher in populations with bigger males, keeping the ratio between male and female size similar among populations (E_1 : 0.88, W_1 : 0.89, W_2 : 0.91; W_4 : 0.83; W_5 : 0.82, W_6 : 0.84).

Geographic Pattern in Bioacoustic Variation

There were significant clinal changes from south to north in most call characters (Fig. 3, Spearman rank correlation coefficient, SVL: $r_s = 0.94$, $P = 0.03$; call duration: $r_s = 0.89$, $P = 0.02$; number of notes: $r_s = 0.89$, $P = 0.02$; dominant frequency: $r_s = 1.00$, $P = <0.001$; call rate: $r_s = -0.77$, $P = 0.07$; rise time: $r_s = 0.89$, $P = 0.02$; note rate: $r_s = -0.18$, $P = 0.70$; $n = 6$ in all cases). Call duration, number of notes per call, and call rise time increased significantly, whereas call repetition rate and dominant call frequency decreased, from south to north.

When grouping populations according to their call characteristics using the discriminant function analysis, the most distant populations

(E_1 and W_6) were placed at the extremes, and were separated by geographically intermediate populations (Fig. 4A). Individuals from different populations were able to be discriminated based on their calls (Wilks' lambda = 0.049, approximate $F_{30,198} = 7.19$, $P < 0.0001$). The calls from different populations of *C. palmatus* were differentiated primarily by Factor 1, which was composed mostly of call duration and number of notes. Although there was some overlap between populations, when pooling all individuals 79.2% were correctly assigned to their population of origin. The highest number of individuals correctly assigned were from populations E_1 , W_4 and W_6 (90% in each case), while individuals from W_1 were poorly assigned (65%).

Geographic Pattern in Genetic Variation

Table 2 represents the estimates of genetic variation for the loci we used. Most of the loci,

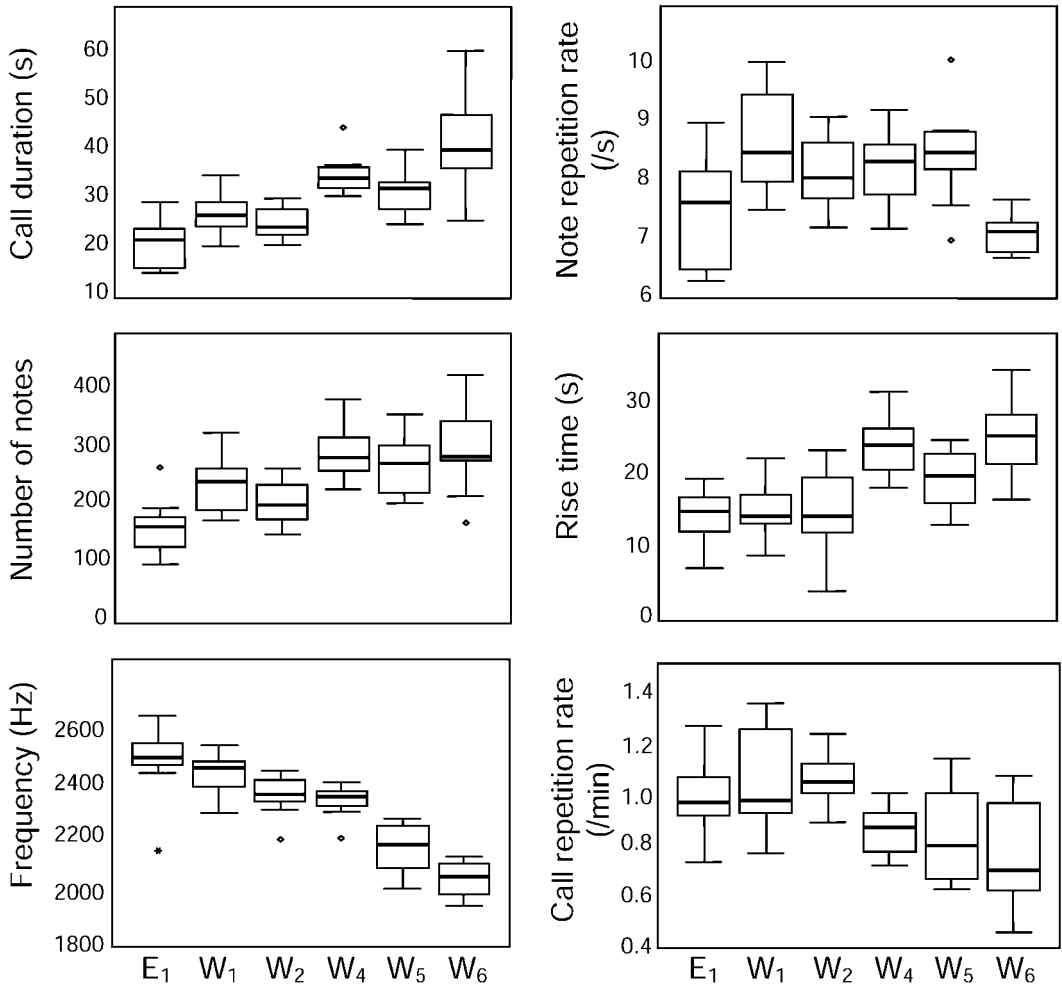


FIG. 3.—Comparison of vocal characteristics of *C. palmatus* calls from six sites. The horizontal bar indicates the median, each block represents 50% of the data, the vertical line the 95% range, and the points are outliers.

24 out of the 30, showed RAPD Nei's genetic variation among populations. Populations differed in which specific loci varied within each one, but all populations had similar amounts of RAPD genetic diversity (ANOVA, $F_{4,145} = 0.753$, $P = 0.557$). The analysis using the UPGMA and Neighbor Joining methods of the entire data set (30 loci per individual) produced a tree which grouped all individuals according to their population of origin. The same topology resulted in the dendrogram generated by both methods, we show the relationships among populations based on the UPGMA analysis in Fig. 5. One of its two main branches contained the population on the

eastern slope only, while the other contained all other populations. The clades supported by higher values were the ones that clustered the populations W₆ (77%), W₄ (81%) and E₁ (70%). Within the western clade there were two groups; one consisted of the northern-most population (W₆), and the second one of the remaining three populations to the south.

When grouping populations according to their genetic structure using the discriminant function analysis, individuals from different populations were correctly discriminated based on their genetic structure (Wilks' lambda < 0.001, approximate $F_{85, 43} = 7.72$, $P < 0.0001$). Most of the variation among

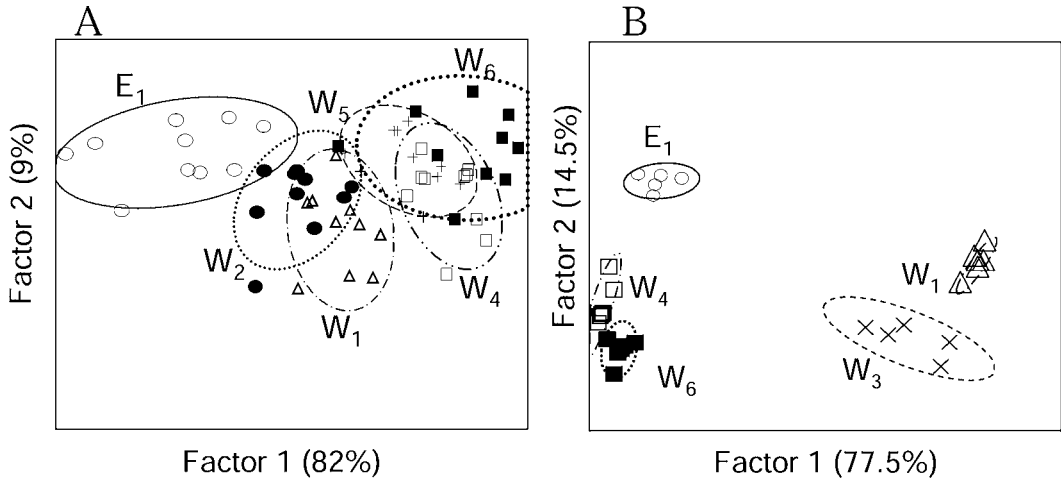


FIG. 4.—Two-dimensional space generated by discriminant function analysis of the advertisement call (A) and genetic structure (B) of *C. palmatus* populations in the Cordillera Oriental. In A, factor 1 accounts for 81.2 % of the variation (canonical correlation = 0.923) while Factor 2 explains 9.5% more of the variation (canonical correlation = 0.634). In B, Factor 1 explains 77.5% of the variation (canonical correlation = 0.995), and Factor 2 explains 14.5% (canonical correlation = 0.974). The confidence ellipses are centered on the centroid of each group. E₁: open circles, W₁: open triangles, W₂: closed circles, W₃: exes, W₄: open squares, W₅: crosses, W₆: filled squares.

populations of *C. palmatus* was explained by Factor 1 (77.5%), but Factor 2 discriminated populations from different slopes separating E₁ from the populations on the other side of the ridge (Fig. 4B). There was no overlap between populations, when pooling all individuals 100% were correctly assigned to their population of origin.

Influence of Geographic Distance and Barriers

Call differences among populations increased with interpopulational geographic distances (Fig. 6A; Mantel test, $g = 3.21$, $Z = 6760.69$, $r = 0.849$, $P = 0.001$). Call differences between populations across the ridge were larger than those along the western slope (Fig. 6A; Partial Mantel test, side of ridge $r = 0.263$, $P = 0.04$; geographic distance $r = 0.788$, $P = 0.0006$; 69.1% variation explained).

We used the RAPD data to calculate the genetic distance among populations and an average rate of effective migration between populations of $N_m = 0.26$ (Table 3). Neither the Nei distance nor the N_m -values showed a significant positive correlation with geographic distance for all possible pairs of populations (Fig. 6B–C; Mantel test, Nei distance: $g = 0.633$, $Z = 228.72$, $r = 0.208$,

$P < 0.05$; N_m -values: $g = -0.298$, $Z = 1008.59$, $r = -0.085$, $P = 0.391$). When only pairs of populations on the western slope were included in the analysis, the genetic distances and N_m -values were significantly correlated with the geographic distance among populations (Fig. 6B–C; Mantel test, Nei distance: $g = 1.799$, $Z = 122.57$, $r = 0.875$, $P = 0.034$; N_m -values, $g = -0.075$, $Z = 623.01$, $r = 0.032$, $P = 0.041$). The highest genetic distances were associated with the geographic distances between pairs that consisted of the eastern population (E₁) with each western population. Partial Mantel tests confirmed a effect of side of the ridge on the genetic distance among populations (Fig. 6B; Partial Mantel test, side of ridge $r = 0.841$, $P = 0.0065$; geographic distance $r = 0.005$, $P = 0.987$; 70.7% variation explained). The N_m -values also covaried with the side of the ridge of the populations contrasted (Fig. 6C; Partial Mantel test side of ridge: $r = -0.657$, $P = 0.029$; geographic distance: $r = -0.292$, $P = 0.422$; 51.76% variation explained).

DISCUSSION

Intraspecific Variation

The analysis of variance revealed statistically significant differences in all call properties,

TABLE 2.—Summary of the genetic variation statistics for all loci in five populations of *C. palmatus* in the Cordillera Oriental. *n*, sample size; *na*, observed number of alleles; *ne*, effective number of alleles; *h*, Nei's gene diversity; *I*, Shannon's Information Index.

Locus	<i>n</i>	<i>na</i>	<i>ne</i>	<i>h</i>	<i>I</i>	Gene diversity (<i>h</i>)				
						<i>E</i> ₁	<i>W</i> ₁	<i>W</i> ₃	<i>W</i> ₄	<i>W</i> ₆
1	29	2	1.489	0.328	0.510	0.000	0.000	0.000	0.000	0.000
2	29	2	1.045	0.070	0.155	0.159	0.159	0.000	0.000	0.000
3	29	2	1.912	0.477	0.670	0.000	0.000	0.465	0.483	0.000
4	29	2	1.524	0.344	0.530	0.000	0.299	0.494	0.488	0.000
5	29	2	1.452	0.311	0.490	0.000	0.159	0.000	0.488	0.488
6	29	2	1.489	0.328	0.510	0.000	0.000	0.000	0.000	0.000
7	29	2	1.489	0.328	0.510	0.000	0.000	0.000	0.000	0.000
8	29	2	1.942	0.485	0.678	0.000	0.000	0.000	0.000	0.000
9	29	2	1.733	0.423	0.614	0.000	0.414	0.189	0.000	0.159
10	29	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	29	2	1.372	0.271	0.442	0.000	0.000	0.494	0.000	0.483
12	29	2	1.890	0.471	0.664	0.000	0.000	0.000	0.000	0.000
13	29	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	29	2	1.993	0.498	0.691	0.414	0.299	0.189	0.000	0.000
15	27	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
16	29	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
17	29	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18	29	2	1.829	0.453	0.646	0.483	0.414	0.349	0.299	0.488
19	29	2	1.462	0.316	0.496	0.000	0.488	0.494	0.000	0.000
20	27	2	1.594	0.373	0.560	0.483	0.500	0.494	0.000	0.000
21	27	2	1.671	0.402	0.591	0.483	0.414	0.494	0.000	0.000
22	29	2	1.942	0.485	0.678	0.000	0.000	0.000	0.000	0.000
23	29	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
24	29	2	1.582	0.368	0.555	0.000	0.000	0.189	0.159	0.000
25	29	2	1.582	0.368	0.555	0.000	0.000	0.189	0.159	0.000
26	29	2	1.390	0.281	0.454	0.000	0.000	0.000	0.299	0.000
27	29	2	1.762	0.432	0.624	0.488	0.000	0.494	0.000	0.681
28	29	2	1.837	0.456	0.648	0.159	0.488	0.349	0.000	0.000
29	29	2	1.183	0.155	0.289	0.483	0.000	0.000	0.000	0.000
30	29	2	1.390	0.281	0.454	0.483	0.000	0.000	0.000	0.676
Mean		1.800	1.486	0.290	0.434	0.121	0.121	0.163	0.079	0.123
SD		0.407	0.331	0.175	0.248	0.202	0.188	0.209	0.161	0.258

except percentage rise time, among the populations. Closer examination using the discriminant function analysis, however, reveals high intrapopulation variation. There is substantial overlapping between calls of individuals from different populations (Fig. 4A). In contrast with the bioacoustic analysis, the genetic data showed less variation within populations and little overlap among them (Fig. 4B).

Variation in note repetition rate within populations of *C. palmatus* was explained by differences in ambient temperature, as previously was found in this species by Lüddecke (1999). As variation in note repetition rate was not related to the social environment (X. Bernal, unpublished data), rate differences between populations may have an ecophysiological component related to the different elevations at which they occur, since mean

annual temperature drops by about 0.6 C for every 100 m increase in altitude (Sturm and Mora-Osejo, 1994). Temperature effects on anuran calls are mostly related to call, note or pulse repetition rate, which depend on cyclic muscle activity (Crespo et al., 1989; Gerhardt, 1988; Girgenrath and Marsh, 1997; Sullivan, 1982). Nevertheless, significant differences in note repetition rate between *C. palmatus* populations remain even when the thermal effect is statistically removed. This difference in note rate suggests that genetic divergence may also be involved.

Clinal variation in call characteristics has been described for several anuran species (Gerhardt, 1982; Hasegawa et al., 1999; Narins and Smith, 1986; Nevo and Capranica, 1985; Ryan et al., 1996; Ryan and Wilczynski, 1991). We found a clinal trend from south to north

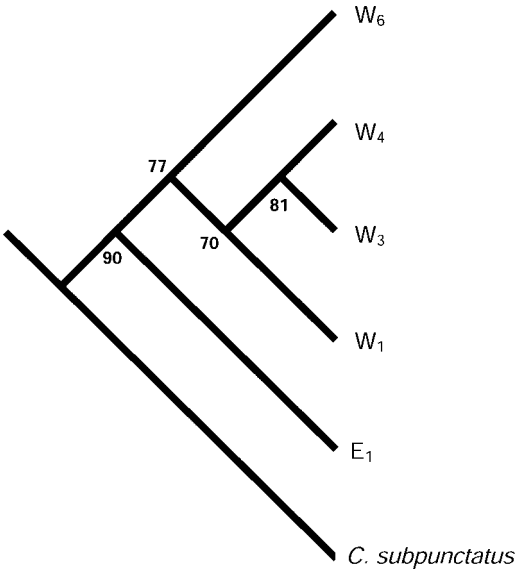


FIG. 5.—UPGMA tree based on RAPD products of five *C. palmatus* populations in the Cordillera Oriental of Colombia. Numbers are bootstrap values as a percentage of 1000 replicates. *Colostethus subpunctatus* was used as an outgroup.

of decreasing call dominant frequency in *C. palmatus*, which may be attributable to a pleiotropic effect of a clinal increase in body size along the same geographical axis. It has been suggested that clinal variation in body size is correlated with clinal variation in moisture because of the advantages enjoyed by bigger size in drier environments (Calhoun and Jameson, 1970; Lee, 1993; Nevo and Capranica, 1985). In the case of *C. palmatus*, although there is clinal variation in moisture along the same axis of variation in size, it goes in the opposite direction. From south to north along the Cordillera Oriental, there is an increase in the amount of moisture combined with an increase in the seasonality of precipitation (Anonymous, 1977; Díaz et al., 1997; Duellman, 1978). Because most populations of *C. palmatus* are found at elevations of 1000–2000 m, where tropical rainfall reaches its highest annual values (Sturm and Mora-Osejo, 1994), and because of the riparian habits of this species (Lüddecke, 1999), it seems unlikely that there are physiological restrictions on water balance, which explain large body size in anuran species living in dry areas.

There was an equivalent cline from south to north in number of notes and call rise time.

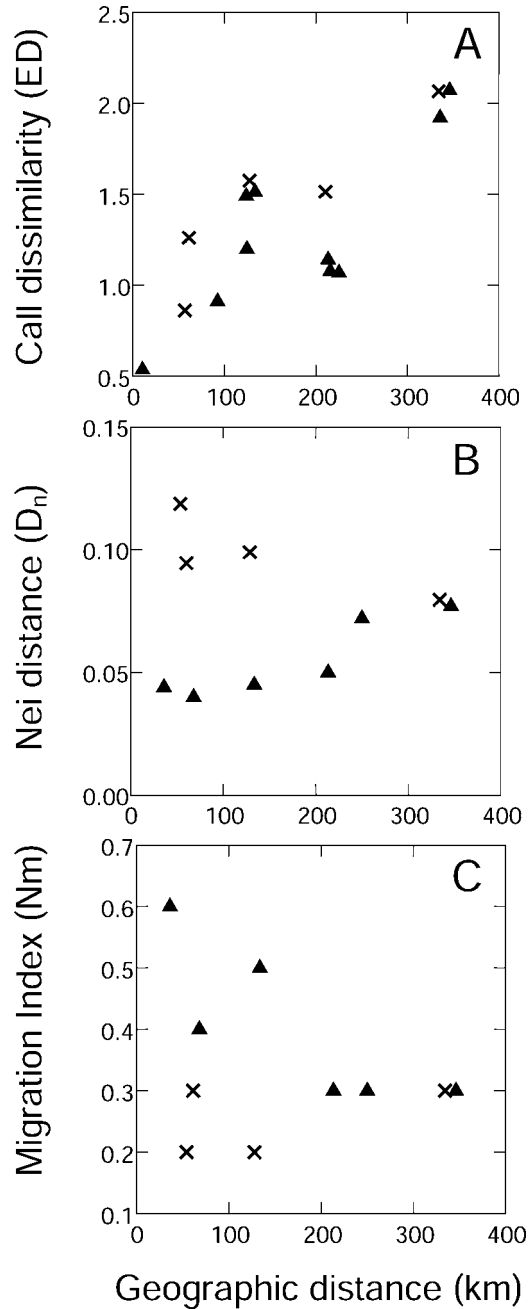


FIG. 6.—Relationships between linear geographic distances separating pairs of *C. palmatus* study sites and the corresponding interpopulational differences in advertisement call characteristics (A), and genetic structure (B–C). The filled triangles correspond to the contrasts between populations on the western slope, and the exes to contrasts between populations across the ridge.

The advertisement calls increased in both of these parameters without increasing note repetition rate producing longer calls in northern populations. The relationship between the cline in body size and number of notes is not clear, however. It is possible that bigger males are able to pump larger volumes of air into their lungs, and therefore produce more notes, but such relation would have to be further investigated.

Influence of Geographic Distance and Barriers

A combination of the two hypotheses, the barrier model and isolation-by-distance model, is necessary to explain the geographic variation of advertisement calls and RAPD products in *C. palmatus*. Call characteristics and genetic distance covaried with geographic location of the populations on the western slope, suggesting a genetic cline that parallels the bioacoustic cline. The high degree of specific genetic structuring within all populations, and the low migration index between populations, suggest that the cline within the latitudinal range occupied by *C. palmatus* is related to restrict horizontal gene flow. Isolation by distance has been shown to cause interpopulational differences in several amphibian species (Green et al., 1996; Lampert et al., 2003; McLister et al., 1991; Scanlan et al., 1980). Among vertebrates, amphibians usually have the highest interpopulational genetic differences (Avice, 1994), probably because of low dispersal capacities (Driscoll, 1998).

Different lines of evidence support the hypothesis that the ridge of the Cordillera Oriental is an altitudinal gene flow barrier between western and eastern populations of *C. palmatus*. Calls of males from the population on the eastern-slope (E_1) are significantly different from calls of males from neighboring populations across the ridge. In fact, the calls from individuals from this population were separated at one end of Factor 1, and were the only ones that did not overlap with calls of individuals from other populations (Fig. 4A). In addition, call dissimilarity between populations at opposite slopes was higher than comparisons between populations on the same side (Fig. 6A). The genetic data also supported the hypothesis of the ridge as a gene flow

TABLE 3.—Estimates of population pairwise genetic distance and migration rate (Nm). Below diagonal: Nei distance, above diagonal: Nm-values.

	E_1	W_1	W_3	W_4	W_6
E_1	—	0.3	0.3	0.2	0.3
W_1	0.095	—	0.6	0.5	0.3
W_3	0.119	0.044	—	0.4	0.3
W_4	0.099	0.045	0.040	—	0.3
W_6	0.080	0.077	0.072	0.050	—

barrier. Population E_1 was located on one of the two main branches of the dendrogram, despite its proximity to W_1 , W_2 and W_3 . Populations were separated based on their side of the Cordillera by Factor 2 in the discriminant function analysis (Fig. 4B). In addition, there was an effect of the ridge on the Nei distance and the migration index between populations (Fig. 6B,C).

The Cocuy ridge probably became effective as a barrier to *Colostethus* frogs late in the course of the rising Cordillera Oriental (Gregory-Wodzicki, 2000), and is assumed to have led to vicariant splitting of a once widespread anuran population (Lynch, 1986). Vicariant events have been proposed to explain geographic variation in traits of several amphibian species in connection with barriers such as mountains, rivers or arid zones (Darda, 1994; García-Paris et al., 1998; Gascon et al., 1996; Grohovaz et al., 1996; Toda et al., 1998; but see Howard et al., 1983). Wide rivers may even be dispersal barriers to anuran populations inhabiting adjacent marshes (Gascon et al., 1996). Populations of *C. palmatus* living on opposite mountain slopes of the eastern chain are undergoing genetic differentiation; the question remains whether they come into contact at the far ends of the mountain chain where the barrier ends. Apparently, the species has not dispersed far enough to the north in order to establish a contact zone in the northern foothills of the mountain chain, and today's aridity of the region seems to constitute a barrier to northward advance of the frogs. There is an additional potential contact zone in the Andalusia pass, a depression of 2000 m altitude about 150 km south of Bogotá. Future fine-grained sampling in that area could confirm whether populations from different slopes come together there.

We found evidence for bioacoustics and genetic differentiation between populations on

opposite slopes of the Cordillera Oriental suggesting lineage differentiation. The limited sampling on the eastern slope in our study, however, restricts us from recognizing two species at present. Additional studies in which more populations are sampled on the eastern slope are necessary. Gathering morphological data and confirming the current records of *C. palmatus* may also be necessary. Rivero and Serna (1995), for instance, proposed that some records assigned to *C. palmatus* can be in fact its sister species, *C. pseudopalmatus*. In addition, there are other Andean species of frogs with extensive ranges in which a similar phenomenon may take place. The ubiquitous *Eleutherodactylus w-nigrum*, for instance, could provide excellent opportunities to examine the effect of barriers and distance among populations in the Andes. Members of other lineages also have representatives on both sides of the Andes (Lynch and Duellman, 1997) offering opportunities to test hypotheses about speciation models in relation to the uplift of the Andes. Examining geographic variation in vocalizations and genetic structure in other widespread Andean species or trans-Andean sister species, would provide comparative data to contrast the influence of geology in the diversification process of the highly rich herpetofauna present in the Andean Cordillera.

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APPENDIX I

Information on the specimens deposited as voucher specimens at the Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia (Bogotá, Colombia) Mesitas (W₁) ICN 44474, ICN 44475; Tena (W₂) ICN 44480, ICN 44481, ICN 44540; Chiquinquirá (W₄) ICN 44484, ICN 44485; Ubaque (E₁) ICN 44482, ICN 44483.

APPENDIX II

Binary matrix for all loci of all individuals of *C. palmatus* in the populations studies in the Cordillera Oriental. For each loci it is shown if a band was present (1) or absent (0). — indicates missing data.

Population	Loci																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
E ₁	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1
	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1
	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1
	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1
	0	1	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0	1	1	1	0	0	0
	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	1	0	0	1	1	1	0	0	1	1	
W ₁	0	0	0	0	0	1	1	1	0	1	1	0	0	1	0	0	1	0	1	1	1	0	0	0	1	1	0	1	1	
	0	0	0	1	0	0	1	1	1	0	1	1	0	1	1	0	0	1	1	0	0	1	0	0	0	1	1	0	1	1
	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	1	1	1	1	1
	0	0	0	0	0	1	1	0	0	1	1	0	1	1	0	0	1	1	0	0	1	0	1	0	0	1	1	1	1	1
	0	1	0	0	1	0	1	1	1	0	1	1	0	0	1	0	0	0	1	—	—	1	0	0	0	1	1	1	1	1
	0	0	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	1	—	—	1	0	0	0	1	1	1	1	1
W ₃	0	0	1	1	0	0	1	1	0	0	0	1	0	0	—	0	0	0	1	1	1	1	0	0	0	1	1	0	1	1
	0	0	1	1	0	0	1	1	0	0	0	0	0	0	—	0	0	0	—	1	1	1	0	0	0	1	1	0	1	1
	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	—	1	1	1	0	1	1	1	1	1	1	1
	0	0	1	1	0	0	1	1	0	0	1	1	0	0	—	0	0	0	1	1	1	1	0	0	1	0	1	0	1	1
	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	1	1	1	1	0	0	1	1	1	0	1	1
W ₄	0	0	1	1	1	0	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1
	0	0	1	1	1	0	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1
	0	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1
	0	0	0	0	1	0	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	1	0	0	0	1	1	1	1	1
	0	0	1	1	0	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	0	0	1	1	1
	0	0	1	1	0	0	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1
W ₆	1	0	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	1
	1	0	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	1
	1	0	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	0	1	1
	1	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1
	1	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	0	0	1
	1	0	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	0	1
<i>C. subpunctatus</i>	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0