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Feeding Habits of *Engystomops pustulatus* (Anura: Leptodactylidae) in Western Ecuador

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Abstract. We describe the diet of adult and juvenile *Engystomops pustulatus* from three populations in the Ecuadorian Chocó. Analysis of the items recovered from the gastrointestinal tracts of 95 individuals showed that *E. pustulatus* has a diverse diet. We identified 52 prey types among 1211 items. The numeric niche breadth, as measured by the Simpson's Index, was 5.37 while the volumetric niche breadth was 5.48. Among juveniles at Cerro Blanco we found a high proportion of small prey items, including termites and ants. Larger items, including snails, coleopterans, and isopods, were most frequent in adults at Patricia Pilar and La Maná. We hypothesize that dietary niche breadth is correlated with the presence of teeth in *Engystomops*. *Engystomops pustulatus* has teeth and has a more generalist diet than the termite and ant specialist species *E. pustulosus*, *E. petersi*, and *E. freibergeri*, which lack teeth.

Keywords. Diet; *Engystomops*; Generalist; Niche Breadth; Prey; Teeth.

Resumen. Describimos la dieta de adultos y juveniles de *Engystomops pustulatus* de tres poblaciones del Chocó Ecuatoriano. El análisis del contenido gastrointestinal de 95 individuos muestra que *E. pustulatus* tiene una dieta diversa. Identificamos 52 tipos de presa entre 1211 presas consumidas. La amplitud de nicho del análisis numérico a nivel de orden, de acuerdo con el índice de Simpson, es de 5.37 mientras que la amplitud de nicho del análisis volumétrico es de 5.48. Encontramos una alta proporción de presas pequeñas, incluyendo termitas y hormigas. Caracoles, coleópteros e isópodos fueron las presas más frecuentes en los individuos adultos colectados en Patricia Pilar y La Maná. Hipotetizamos que la amplitud del nicho alimenticio está correlacionada con la presencia de dientes en *Engystomops*. *Engystomops pustulatus* tiene dientes y su dieta es más generalista que la de las especies especializadas en hormigas y termitas *E. pustulosus*, *E. petersi* y *E. freibergeri* las cuales carecen de dientes.

INTRODUCTION

Feeding habits define the trophic role of animals on ecosystems and give information about natural history and behavior (Díaz and Ortiz, 2003). Most adult amphibians eat invertebrates, especially insects, and most species are considered generalists because their diets reflect prey availability according to their own size (Duellman and Trueb, 1986; Parmelee, 1999; Hirai and Matsui, 2000). However, some species have evolved specializations (Parmelee, 1999). Few studies report the evolution of diet among species in frogs (Toft, 1995), and it appears that dietary change over time tends towards specialization (Fabrezi, 2001). A specialist diet excludes a majority of the available prey types; a generalist diet includes most available prey types (Toft, 1995; Parmelee, 1999; Hirai and Matsui 2000; Díaz and Ortiz, 2003).

Some studies have shown that there is a correlation between diet and feeding behavior (Emerson, 1985; Duellman and Lizana, 1994) and between types of prey. Prey are grouped according to hardness, size, and speed, as well as predator morphology, such as the presence and shape of teeth, jaw closure, tongue and skull shape (Emerson, 1985; Wells, 2007). Studying the evolution of such adaptations first requires an adequate description of diet composition.

A group that could offer insight into the evolution of feeding adaptations is the genus *Engystomops*. These

Neotropical frogs are divided into two sister clades that have received the unranked names of Duovox and Edentulus (Ron *et al.*, 2006). As its name implies, Edentulus is characterized by the absence of teeth, and previous dietary studies showed that they are termite specialists (Menéndez, 2001; Parmelee, 1999; Ryan, 1985; Duellman, 1978). Because teeth are present in Duovox, *Physalaemus* (*Engystomops* sister group; Cannatella *et al.*, 1998), and *Edalorhina*, the loss of teeth in Edentulus appears to be a derived state (Fig. 1). The variation in the presence of teeth makes *Engystomops* ideal for exploring hypotheses on the evolution of teeth in relation to diet specialization.

Herein we analyze the diet of *Engystomops pustulatus*—a Duovox species—and compare it to previously published diet data for three Edentulus species. We explore the hypothesis that loss of teeth in this group is correlated with dietary specialization by comparing the dietary niche breadths of species. We predict that the niche breadth of *E. pustulatus*, which has teeth, will be broader than the niche breadths of the three other species that lack teeth.

MATERIALS AND METHODS

Engystomops pustulatus Shreve (1941) is a leptodactylid frog distributed in the Ecuadorian Chocó bioregion

in the lowlands of western Ecuador. Its occurs in disturbed areas, secondary forests, and deciduous forests (Ron *et al.*, 2010, 2006; Sierra, 1999). These frogs are characterized by the deposition of eggs in foam nests built by males. They reproduce during the rainy season between December and April; throughout this period, males aggregate and call from temporary ponds to court females and mate. We analyzed the gastrointestinal contents of 40 adult males from Patricia Pilar (Los Ríos Province, 0.53717°S, 79.37071°W, 200 m above sea level), 14 adult males from La Maná (Cotopaxi Province, 0.94387°S, 79.24429°W, 193 m above sea level) and 41 juveniles from Bosque Protector Cerro Blanco (BPCB; Guayas Province, 2.17999°S, 80.0197°W, 281 m above sea level). At Patricia Pilar and La Maná, we collected frogs at night from reproductive choruses at temporal ponds in artificial open areas; at Cerro Blanco, we did not find choruses because

of dry conditions. Consequently most individuals were collected along trails in secondary forest or artificial open areas. Collections took place between February and April 2008 at Patricia Pilar, December 2003 at La Maná, and May 2008 at Cerro Blanco when frogs were abundant.

To maximize the number of recognizable dietary items, all frogs were euthanized using Roxicaine (anesthetic spray) and fixed in 10% formalin shortly after collection (less than 3 hours). After fixation, specimens were preserved in 75% ethanol. Sex of the specimens was determined by the presence of vocal sac folds in the gular region and nuptial pads in males. Reproductive stage (ontogeny) was identified by direct examination of the gonads. Analyzed specimens and gastrointestinal contents are deposited at the Museo de Zoología of Pontificia Universidad Católica del Ecuador (QCAZ) and are listed in Appendix I.

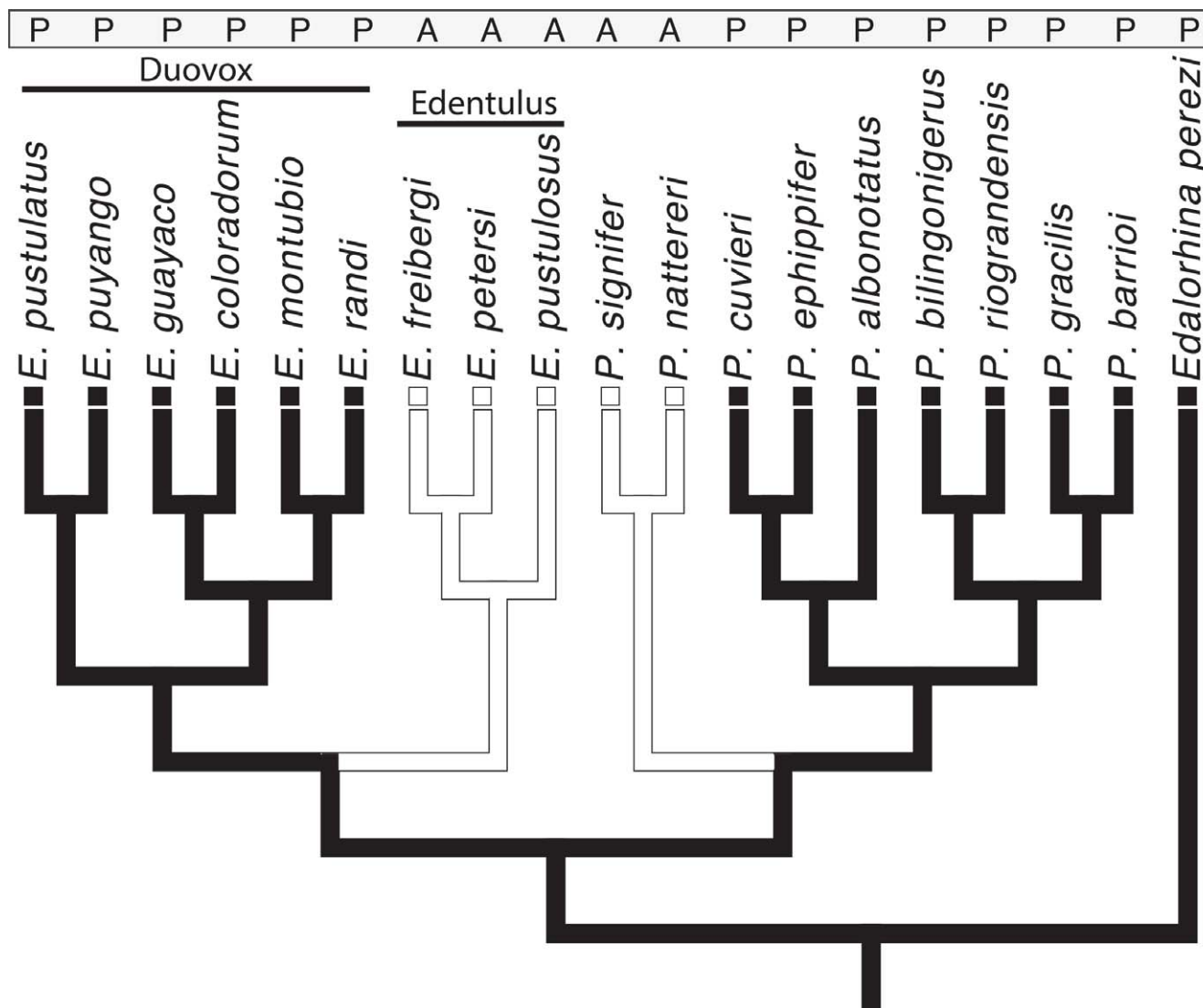


Figure 1. Phylogeny showing the distribution of maxillary and premaxillary teeth among species of *Physalaemus*, *Engystomops*, and *Edalorhina perezii*. Ancestral character state reconstruction is based on maximum parsimony. Phylogeny based on Ron (2010) and Pyron and Wiens (2011); character states from Cannatella *et al.* (1998), Lynch (1970; 1971), and Nascimento *et al.* (2005). P = teeth present; A = teeth absent; black branches = presence; white branches = absence.

Contents of the stomach and intestine were analyzed separately under an STEMIDW4 (32X) microscope following Parmelee (1999). All items were identified to the lowest taxonomic level possible—generally order or family—following Triplehorn and Johnson (2005) taxonomy. However, niche breadth analyses were based only on order-level categories. Vegetable material (only present in few individuals) and highly digested prey items (unidentifiable) were not considered in the analyses.

Prey length and width were measured with an electronic calliper (to the nearest 0.1 mm) to estimate prey volume with the formula for a prolate spheroid (Parmelee, 1999):

$$V = \frac{4}{3} \pi \left(\frac{\text{length}}{2} \right) * \left(\frac{\text{width}}{2} \right)^2$$

Legs, antennae and ovipositors were not measured to estimate volume, but were included in prey counts.

We quantified the diet diversity of *Engystomops pustulatus* by calculating niche breadth with Simpson's reciprocal index (Simpson, 1949). This index is maximized when the level of consumption is the same for all prey categories and minimized when only one category is consumed as the principal resource. Simpson's index considers the importance of the most dominant species (Simpson, 1949; Moreno, 2000).

The formula for Simpson's index is:

$$B = \frac{1}{\sum_{i=1}^n p^2}$$

Where, p is the proportion of the prey category consumed (numeric or volumetric respectively), i is the prey category consumed, and n is the total number of prey categories. Because prey availability in the environment was not evaluated, n corresponds to the total number of items consumed by each population. High values of B represent a more generalist diet (maximum value is the number of diet categories present) and 1 represents a specialist diet (Ávila *et al.*, 2008). To compare our niche breadth results with those of *Edentulus*, we estimated the Simpson index of *Engystomops pustulosus*, *E. freibergi* and *E. petersi* using diet data reported by Duellman (1978), Menéndez (2001), Ryan (1985), and Parmelee (1999). Indices were obtained from proportions of the number of prey using a similar methodology to the present study, as well as the same level of prey categories (order-level).

To compare diet composition between populations of *Engystomops pustulatus*, both volumetrically and numerically, we applied a likelihood ratio G^2 test; we did not use the chi-squared test because it cannot be applied when one or more category has sample size > 5 (Sokal and

Rohlf 1995). Because the G^2 test requires values > 5 in at least 80% of the contingency table cells, we grouped values in order-level categories. Categories were excluded if they had less than 15 prey items in the sum of the populations being compared. Populations were represented as rows in contingency tables and taxonomic categories of prey were represented as columns. Analyses were made using JMP software, Version 7. SAS Institute Inc., Cary, NC, (1989–2007).

RESULTS

We identified 1211 items from the gastrointestinal contents of three populations of *Engystomops pustulatus*. The most frequent groups in the diet were arthropods (insects, arachnids, millipedes and centipedes) and gastropods. They were composed of 52 prey categories of 18 orders. We found 41 categories of 17 orders at BPCB, 27 (15 orders) at Patricia Pilar, and 11 (7 orders) at La Maná. The most abundant preys consumed by adults at La Maná and Patricia Pilar were ants (49% and 28%, respectively), followed by cicadas at La Maná (7%) and snails at Patricia Pilar (17%) (Table 1). Diet composition differed significantly between both populations ($G^2 = 18.7$, $p = 0.002$). Diet was markedly different between Patricia Pilar and BPCB ($G^2 = 307.9$, $p < 0.001$). At BPCB, where all individuals were juveniles, the most abundant prey were termites (35%) and ants (30%) (Table 1). Because of low cell values in the G^2 test contingency table, we could not compare La Maná with BPCB. The most abundant prey item at BPCB (termites) was absent in the diet at La Maná.

At Patricia Pilar the most voluminous prey category was isopods (51%), followed by snails (21%); at La Maná the most voluminous categories were beetles of the Elateridae family (68%) and ants (14%); at BPCB the most voluminous preys were termites (48%), followed by snails (11%) (Table 1). Both categories comprised 59% of the total volume (Table 1). There were significant differences in diet composition between BPCB and Patricia Pilar ($G^2 = 1233.9$, $p < 0.001$).

We found 17 empty stomachs, 10 in Patricia Pilar (25% of the individuals examined), 4 in La Maná (28%), and 3 in BPCB (7%).

The numeric niche breadth of *Engystomops pustulatus* (order-level categories) was 6.26 at Patricia Pilar, 4.3 at BPCB, and 3.41 at La Maná; the aggregate breadth from the combined localities was 5.37. The volumetric niche breadth was 4.29 at Patricia Pilar, 3.71 at BPCB, and 1.66 at La Maná; the aggregate breadth was 5.48. The numeric niche breadths of the toothless species of *Engystomops* were lower than those of *E. pustulatus*: 1.01 for *E. petersi* ($n = 32$), 3.15 for *E. pustulosus* ($n = 29$), and 1.02 for *E. freibergi* ($n = 7$). The most abundant type of prey in the three toothless species was termites.

Table 1. Diet of *Engystomops pustulatus* adults at Patricia Pilar, Los Ríos and La Maná, Cotopaxi and subadults at Bosque Protector Cerro Blanco, Guayas (BPCB). Number and volume of prey category and niche breadth at each locality are detailed.

Prey Category		Patricia Pilar				BPCB				La Maná			
		Number		Volume		Number		Volume		Number		Volume	
Order	Family	#	%	(mm ³)	%	#	%	(mm ³)	%	#	%	(mm ³)	%
Araneae	indeterminate	8	2.97	33.29	6.37	24	2.72	25.53	2.62	5	8.20		
Blattodea	indeterminate	1	0.37			1	0.11						
Chilopoda	indeterminate	8	2.97	70.22	13.44	6	0.68						
Coleoptera	Anthicidae	1	0.37	1.02	0.20								
	Carabidae	3	1.12			3	0.34	3.65	0.37				
	Chrysomelidae					2	0.23	1.09	0.11				
	Coccinellidae					3	0.34	1.45	0.15				
	Colydiidae	1	0.37			5	0.57						
	Cucjoidea	1	0.37			2	0.23	0.49	0.05				
	Curculionidae	1	0.37			5	0.57	0.65	0.07	1	1.64	1.36	8.28
	Dermeestidae					1	0.11	5.87	0.60				
	Dityscidae					2	0.23	0.26	0.03				
	Elateridae	5	1.86	2.30	0.44	2	0.23	1.61	0.17	1	1.64	11.12	67.64
	Erotilidae	2	0.74										
	Histeridae					1	0.11	1.22	0.13				
	indeterminate	12	4.46			7	0.79			1	1.64		
	Leiodidae	1	0.37										
	Nitidulidae	1	0.37	1.26	0.24	1	0.11	1.18	0.12				
	Pselaphidae					1	0.11	0.23	0.02				
	Scarabeidae	2	0.74	1.99	0.38					1	1.64		
Scolytidae	1	0.37											
Staphylinidae	1	0.37			7	0.79	41.54	4.26					
Dermaptera	indeterminate	1	0.37			5	0.57						
Diplopoda	indeterminate	4	1.49			3	0.34	1.89	0.19				
Diptera	indeterminate	4	1.49	0.98	0.19	6	0.68	10.09	1.03				
	larvae					20	2.27						
	Tipulidae	1	0.37	1.08	0.21	1	0.11	0.28	0.03				
Gasteropoda	indeterminate	46	17.10	108.04	20.67	36	4.09	109.7	11.24	4	6.56		
Hemiptera	Anthocoridae					13	1.48	11.30	1.16	2	3.28		
	Cicadellidae	10	3.72	6.54	1.25	33	3.75	21.40	2.19	4	6.56	0.61	3.69
	Coccidae					1	0.11	0.30	0.03				
	indeterminate	9	3.35			10	1.14	4.32	0.44	1	1.64		
	Largidae	2	0.74							2	3.28		
	Lygaeidae					1	0.11						
	Membracidae					1	0.11						
	Miridae	3	1.12			4	0.45	4.39	0.45				
	Reduviidae	4	1.49			3	0.34						
	Tingidae	2	0.74			1	0.11						
Hexapoda	Collembola					42	4.77	6.22	0.64				
Hymenoptera	Chalcidoidea	2	0.74	2.13	0.41	6	0.68	3.91	0.40				
	Formicidae	76	28.25	25.98	4.97	262	29.74	67.83	6.95	30	49.18	2.35	14.31
	indeterminate	1	0.37										
Ixodida	Argasidae	7	2.60	0.74	0.14	7	0.79	1.08	0.11	6	9.84	0.93	5.68
	Ixodidae	21	7.81	2.02	0.39	13	1.48	3.02	0.31	1	1.64	0.07	0.41
Lepidoptera	indeterminate	1	0.37										
	larvae	4	1.49			15	1.70	95.19	9.75	2	3.28		
Isopoda	indeterminate	17	6.32	265.03	50.71	3	0.34	10.83	1.11				
Isoptera	indeterminate					312	35.41	468.83	48.04				
Odonata	larvae	2	0.74										
Orthoptera	Tetrigidae	3	1.12			1	0.11	30.04	3.08				
	Tettigoniidae					2	0.23	39.14	4.01				
Pseudoescopionida	indeterminate					7	0.79	1.35	0.14				
Niche breadth at order level		6.26		4.3		4.3		3.71		3.41		1.66	

DISCUSSION

Our results show that *Engystomops pustulatus* has a diverse diet. We found 52 prey types among the three populations sampled. The numeric niche breadth in *E. pustulatus* is broader compared to the toothless species: *E. petersi* in Amazonian Ecuador (Duellman, 1978; Menendez, 2001), *E. freibergi* in Amazonian Peru, (Parmelee, 1999), and *E. pustulosus* in Panamá (Ryan, 1985).

Feeding habits correlate with morphology (Emerson, 1985; Das and Coe, 1994; Wells, 2007), and teeth seem to strongly influence the type of prey captured. Teeth are characteristic of species that feed on large and hard prey and tend to be absent in species that feed on small and soft prey, especially termites and ants—like microhylids, bufonids, and some poison frogs (Kikkawa and Dwyer, 1992; Das and Coe, 1994; Parmelee, 1999; Clark *et al.*, 2005). Our results are consistent with this pattern because they show that *Engystomops pustulatus* has a more generalist diet than the species lacking teeth, *E. freibergi*, *E. petersi*, and *E. pustulosus*.

In general, tooth reduction or absence is considered a derived character state (Fabrezi, 2001; Wiens, 2011), and the distribution of teeth in *Engystomops* and the closely related genera *Physalaemus* and *Edalorhina* is congruent with that pattern. Reconstruction of tooth presence on the most complete available phylogeny of *Engystomops* and *Physalaemus* suggests that the absence of teeth is derived in Edentulus (Fig. 1). If diet and dentition are correlated, the specialist diet of Edentulus must be derived as well. This scenario is confirmed by our results and diet data from the literature. Similarly to *E. pustulatus*, *Physalaemus* species have a generalist diet, although with a tendency to feed on termites or ants (Caldwell and Vitt, 1999; Da Rosa *et al.*, 2002; Santana and Juncá, 2007; Araujo *et al.*, 2009). Thus, under the most parsimonious scenario, the ancestor of *Engystomops* likely had a generalist diet, and the extreme diet specialization in Edentulus is derived. These character reconstructions, however, are tentative because the phylogeny of *Physalaemus* has poor taxon sampling, and diet is unknown in most Edentulus and *Physalaemus*. This information will be necessary to test the hypothesis of co-evolution between teeth and diet specialization using phylogenetic comparative methods.

Termites represent 100% of the diet of *Engystomops petersi* and 99% of *E. freibergi*, and ants and termites represent 81% of the diet of *E. pustulosus* (Ryan, 1985). Even though termites and ants were among the most abundant prey items in the diet of *E. pustulatus*, individually they represent less than half of the adult diet. Ryan (1985) reports few categories consumed by *E. pustulosus*. In contrast, Gonzales-Duran *et al.* (2012) report 11 prey categories at a population in Reserva Rio Manso (Colombia); however, ants and termites were the most abundant preys at Rio Manso while the other categories were scarce. For

this reason, Gonzales-Duran *et al.* (2012) suggest that the other categories may be accidentally ingested, and they conclude that *E. pustulosus* has a specialized diet. On the contrary, in *E. pustulatus* we observed categories other than termites and ants to be well represented, such as Coleoptera, Hemiptera, or Gasteropoda. For this reason, we conclude that the diet of *E. pustulatus* is more generalist than the diet of its congeners from clade Edentulus.

Analyses of diet composition show that *Engystomops pustulatus* has a diverse diet with significant differences between populations, yet the cause of those differences is unclear. Differences in diet composition between BPCB and Patricia Pilar, for example, could result from ontogenetic, seasonal or populational differences. Our sampling strategy does not allow discrimination among these alternatives because differences in feeding habits related to life stage, locality, and season are well known in anurans (Duellman and Lizana, 1994; Toft, 1995; Wells, 2007). We also observed that a large number of adult males from the breeding chorus had empty stomachs compared with juveniles collected in BPCB. This finding is consistent with previous studies showing that males stop foraging while they attend choruses during reproductive season (Menin *et al.*, 2005; Ryan, 1985).

In conclusion, the diet of *Engystomops pustulatus* is more generalist than that of its congeners that lack teeth. Further species sampling for diet and teeth-state in *Engystomops* and *Physalaemus* is required to rigorously test this correlation.

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APPENDIX I

Specimens examined for diet analyses. All specimens are deposited in the Museum of Zoology at Pontificia Universidad Católica del Ecuador, Quito (QCAZ).

Ecuador, Provincia Los Ríos, Patricia Pilar: 38377, 38378, 38379, 38380, 38381, 38382, 38383, 38384, 38385, 38386, 38387, 38388, 38389, 38390, 38391, 38392, 38393, 38394, 38395, 38396, 38397, 38398, 38399, 38400, 38401, 38402, 38403, 38404, 38405, 38406, 38407, 38408, 38409, 38410, 38411, 38412, 38413, 38414, 38415, 38416.

Ecuador, Provincia Guayas, Bosque Protector Cerro Blanco: 38336, 38337, 38338, 38339, 38340, 38341, 38342, 38343, 38344, 38345, 38346, 38347, 38348, 38349, 38350, 38351, 38352, 38353, 38354, 38355, 38356, 38357, 38358, 38359, 38360, 38361, 38362, 38363, 38364, 38365, 38366, 38367, 38368, 38369, 38370, 38371, 38372, 38373, 38374, 38375, 38376.

Ecuador, Provincia Cotopaxi, La Maná: 26152, 26153, 26154, 26155, 26156, 26157, 26158, 26159, 26160, 26161, 26162, 26163, 26164, 26165.