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SPECIES DIVERSITY AND CONSERVATION STATUS OF AMPHIBIANS IN MADRE DE DIOS, SOUTHERN PERU

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ABSTRACT.—This study focuses on amphibian species diversity in the lowland Amazonian rainforest of southern Peru, and on the importance of protected and non-protected areas for maintaining amphibian assemblages in this region. We compared species lists from nine sites in the Madre de Dios region, five of which are in nationally recognized protected areas and four are outside the country's protected area system. Los Amigos, occurring outside the protected area system, is the most species-rich locality included in our comparison. Overall, species similarity is relatively high among various localities in Madre de Dios. Among the 114 species recorded in Madre de Dios, nine (7.9%) have only been recorded on land outside of protected areas. This number emphasizes the need to conserve additional sites in the region, especially in the face of rapid habitat destruction. In addition, preliminary results from comparisons of species richness and abundance at the Los Amigos site suggest that forest type may affect the species composition, abundance, and distribution of amphibians at the local scale (area < 1000 ha). These results have wider implications for reserve design and habitat conservation decisions. We also present data on the conservation status of the amphibians of Madre de Dios according to IUCN and CITES categories.

Key Words.—Amazonia; amphibians; beta diversity; frogs; habitat; protected areas

INTRODUCTION

The primary goals of creating protected areas are to ensure the long-term functioning of ecosystems and representation of biodiversity in a region (Margules and Pressey 2000). However, a great number of species reside outside the boundaries of protected areas (also referred to as preserves in this paper). This is especially true in tropical countries, where biodiversity is high, environmental law enforcement is difficult, and forest habitat loss is widespread (Rodrigues et al. 2003; Brooks et al. 2004). Unprotected lands near preserves are crucial resources for maintaining species diversity in these preserves because they buffer core areas of preserves and they may support healthy populations of native biota (DeFries et al. 2005; Hansen and DeFries 2007). Comparing species richness between protected and non-protected sites allows us to assess the effectiveness of preserves in the conservation of biodiversity. Low species richness is commonly assumed for non-protected sites, especially in areas with high levels of anthropogenic disturbance (Ernst and

Rödel 2005). However, there are large tracts of unprotected land worldwide that exhibit high species richness (Brooks et al. 2004; Rodrigues et al. 2004). Thus, it is reasonable to surmise that relatively undisturbed, non-protected sites may exhibit similar species richness to that of protected areas (Rodrigues et al. 2003).

The lowland rainforests of southern Peru offer an excellent setting to evaluate these questions. The Peruvian government protects about half of the Madre de Dios region (a region in Peru is a land delineation analogous to a state in the United States), which has an area (85,300 km²) approximately 1.5 times the size of Costa Rica. The other half is not protected and includes both forested and non-forested lands; the latter include converted lands with different levels of use (Oliveira et al. 2007). Current threats to the region's biodiversity include habitat loss associated with human settlement of the area, gold mining, and other anthropogenic impacts (e.g., illegal logging, slash-and-burn agriculture) that are expected to increase as a result of the paving of the interoceanic highway that will connect Brazil to the Pacific

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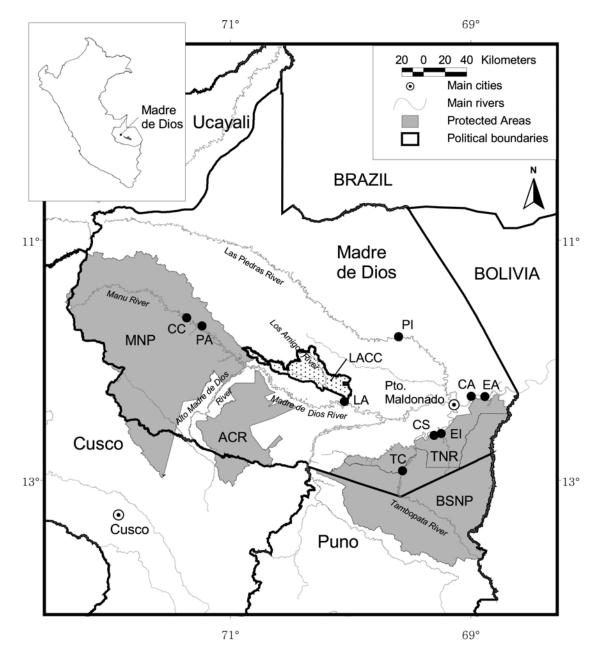


FIGURE 1. Map of Madre de Dios region depicting the natural protected areas (gray) that belong to Peru's National System of Protected Areas (SINANPE) and the nine lowland sites included in this study. Dotted area is Los Amigos Conservation Concession (LACC), which does not belong to SINANPE. Inset depicts the location of Madre de Dios in southeastern Peru. Site abbreviations: CA = Cuzco Amazónico, CC = Cocha Cashu, CS = Centro Sachavacayoc, EA = Eco Amazonia, EI = Explorer's Inn, LA = Los Amigos, PA = Pakitza, PI = Piedras, TC = Tambopata Center.

coast of southern Peru (Oliveira et al. 2007). Researchers have increased the amount of information on the biodiversity of Madre de Dios during the last three decades, both in nationally-recognized preserves (e.g., Terborgh 1983; Erwin 1985; Emmons 1987; Foster 1990; Gentry and Terborgh 1990; Lamas et al. 1991) and outside these preserves (e.g., Goulding et al. 2003; Phillips et al. 2003; Pitman 2006). In Peru, the term "protected area" refers to a nationally-recognized area that belongs to a system of natural protected areas (Sistema Nacional de Areas Naturales Protegidas – SINANPE). The most thoroughly studied site within one of these preserves is Cocha Cashu Biological Station, located in Manu National Park, though it only represents a portion of the total variation in biodiversity found in Madre de Dios. Some unprotected sites may exhibit similar species richness and more terrestrial habitats than those found within preserves (Phillips et al. 2003; Pitman 2006). Protecting the biodiversity of the Madre de Dios region makes assessment of nonprotected sites critical to the conservation of the regional biota. For example, five new species of frogs were described from a non-protected site used for research and ecotourism activities (Duellman 2005).

Amphibians are model organisms for studies on variation in species richness and composition in protected and non-protected sites. Many species of amphibians are particularly susceptible to habitat degradation and other environmental factors (Blaustein et al. 1994; Pearman 1997; Young et al. 2001). Their small size and high relative abundance, compared to most other vertebrates, allow researchers to quantify species richness from localized areas using standardized field techniques (Heyer et al. 1994). The regional (gamma) diversity of amphibians in Madre de Dios is fairly well known (Duellman 2005), but additional surveys in new sites will allow us to determine the extent to which regional diversity is influenced by species turnover among sites (i.e., beta-diversity) and to evaluate the role of protected versus non-protected sites.

Previous researchers inventoried amphibians at seven lowland sites in Madre de Dios (Rodríguez and Cadle 1990; Duellman and Salas 1991; Morales and McDiarmid 1996; Doan and Arizabal Arriaga 2002; and see summary in Duellman 2005). Five of these sites (Cocha Cashu Biological Station, Centro Sachavacayoc, Explorer's Inn, Pakitza, and Tambopata Research Center) are inside of preserves and two (Cusco Amazónico and Eco Amazonia) occur in non-protected sites (Fig. 1). We present results of amphibian inventories at two additional non-protected sites (Los Amigos Research Center [herein, referred to as Los Amigos], and Las Piedras Biodiversity Station [herein referred to as Las Piedras]). We combine data from Los Amigos and Las Piedras with data from previous inventories to provide a more complete species list and description of the amphibian diversity found in the Madre de Dios region. Los Amigos and Las Piedras fill a gap of more than 200 km among the previously studied sites (Fig. 1).

Our objectives were: (1) to evaluate the species composition of amphibian assemblages within Madre de Dios; (2) to assess and compare amphibian species composition inside and outside of protected areas; and (3) to assess the conservation status of amphibians in Madre de Dios according to categories established by the International Union for the Conservation of Nature (IUCN) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

MATERIALS AND METHODS

Study sites.—The Los Amigos Research Center (12°34′ S, 70°06′ W; 270 m elevation) is in Manu province, Madre de Dios region, southern Peru. The area we surveyed around Los Amigos covers

approximately 500 ha and it is privately owned by the Amazon Conservation Association, a non-governmental organization that also administers a conservation concession totaling 145,918 ha (Pitman 2006). The Las Piedras Biodiversity Station (12°07' S, 69°54' W; 300 m elevation) is in the Tambopata province, Madre de Dios region, southern Peru. Las Piedras is an ecotourism concession that covers ca. 5000 ha; however, we surveyed less than 500 ha of this area. Both sites exhibit a heterogeneous landscape of terrestrial and aquatic habitats characteristic of the western Amazonian Terrestrial forested habitats include nonlowlands. flooded terra firme, floodplain, palm swamp, and bamboo. Bamboo forest is not found at Las Piedras. Aquatic habitats include temporary ponds, temporary and permanent streams, rivers, and oxbow lakes (Goulding et al. 2003). The annual rainfall is 2700-3000 mm and is seasonally distributed. A dry season lasts about six months, extending from May to October (rainfall < 100 mm/month) and is slightly cooler (21-26 °C) than the wet season (Pitman 2006). The geographic location of Los Amigos suggests that rainfall patterns and temperature resemble those at Las Piedras. The remaining seven lowland sites in Madre de Dios are Cuzco Amazónico, Cocha Cashu Biological Station, Centro Sachavacayoc, Eco Amazonia, Explorer's Inn, Pakitza, and Tambopata Center (Fig. 1). Information for these sites is summarized in Duellman (2005); specific details are available in Rodríguez and Cadle (1990), Duellman and Salas (1991), Rodríguez (1992), Morales and McDiarmid (1996), and Doan and Arizabal Arriaga (2002).

Data collection at Los Amigos and Las Piedras.—We spent 711 person-days at Los Amigos from 2001 – 2007. We conducted field work at Los Amigos in seven sampling periods: 26 August – 18 September 2001 (dry season), 25 January – 22 February 2002 (wet season), 18 January – 5 May 2003 (wet season), 5 November 2004 – 6 April 2005 (wet season), 5 July – 13 August 2005 (dry season), 13 July – 7 August 2006 (dry season), and 20 January – 18 April 2007 (wet season). Field work at Las Piedras encompassed 102 person-days during two sampling periods: 13 June – 20 July 2003 (dry season) and 21 November – 15 December 2003 (wet season).

We conducted field work at both sites following standard methods for measuring and monitoring amphibian diversity (Heyer et al. 1994). We used visual encounter surveys (VES) to determine the species richness and relative abundance of understory and leaf-litter amphibians in the four major forest types (Crump and Scott 1994). We used leaf-litter plots to determine the species richness and density of leaf-litter amphibians (Jaeger and Inger 1994). We used 10 x 10 m plots only during the 2003 sampling period. We used pitfall traps

with drift fences to record ground dwelling amphibians not commonly found using the other methods (Corn 1994). We conducted all sampling at night, usually between 1900 h and 0130 h, by two or three observers with headlamps. We used nocturnal VES and nocturnal leaf-litter plots because most amphibians are nocturnal and these methods are more effective compared to diurnal sampling (Doan 2003; Donnelly et al. 2005a). Two sampling periods at Los Amigos involved pitfall trapping, the dry seasons of 2005 and 2006. We checked pitfall traps daily for one month. We captured, identified, and measured (snout-vent length, SVL) and then released all amphibians as encountered. To obtain a physical record of the amphibian diversity of the study sites and to verify the species' identify in cases where field identification was not possible, we collected one to four voucher specimens per species. Specimens were fixed in a 10 % formalin solution (1 - 5 days), labeled for identification, and then stored in 70 % ethanol. We deposited voucher specimens (of all species recorded) in the herpetological collection at the Museum of Natural History, Universidad Nacional Mayor de San Marcos, in Lima, Peru. Species identity was verified using the herpetological collection at this museum, species descriptions, and field guides. In some cases, we verified species identity by consultation with specialists (see Acknowledgments). Catalogue numbers are available upon request and are also available in the museum's herpetology collection catalogue.

Species lists.-We used the two most recent publications on herpetofaunal communities of southern Peru (Doan and Arizabal Arriaga 2002; Duellman 2005) and our previously unpublished data from Los Amigos and Las Piedras to build a more complete list of amphibians inhabiting the lowlands (< 500 m) of Madre de Dios region. We combined previously published species lists (Doan and Arizabal Arriaga 2002; Duellman 2005) to analyze amphibian diversity. However, as did Duellman (2005), we chose not to include previously recorded but unvouchered records (Pristimantis [formerly Eleutherodactylus] martiae, Leptodactylus fuscus, Phyllomedusa tarsius, Scinax cruentommus, and Lithobates [formerly Rana] palmipes) from Doan and Arizabal Arriaga (2002). We taxonomically organized species according to the most recent information available (Frost et al. 2006; Grant et al. 2006; Heinicke et al. 2007: De la Riva et al. 2008).

Statistical analyses.—We estimated similarity among sites in Madre de Dios with the Coefficient of Biogeographic Resemblance (CBR; Duellman 1990). We chose this measure to compare our findings with previously published reports that also included CBR values to describe the variation in amphibian composition of Madre de Dios (Doan and Arizabal

Arriaga 2002; Duellman 2005). We calculated CBR with the following formula (Duellman 1990): CBR = 2C $/(N_1 + N_2)$, where C is the number of species present in both of a pair of sites, N_1 is the number of species present in the first site, and N₂ is the number of species present in the second site. A value of zero means that there are no shared species between two sites; in contrast, a value of one means that both sites have identical species composition. Although sampling effort varied across sites and a small number of species have probably not been recorded at undersampled sites, we consider that our synthesis of existing data (combining published species lists and our own dataset) provides a reliable estimate of regional amphibian species richness inside and outside preserves. Ideally, we would have sampled all sites using the same methods, but because of various limitations this was not possible and we worked with existing data (published species lists from seven sites and our own dataset from Los Amigos and Las Piedras). This approach has also been used by other researchers to compare multiple inventory data from lowland amphibian faunas in Amazonia (Azevedo-Ramos and Galatti 2002; Duellman 2005) and other regions (Veith et al. 2004; Donnelly et al. 2005a, 2005b). In these studies, researchers compared sites where amphibian inventories differed in terms of sampling effort but regardless, they were able to provide a more complete summary of the regional amphibian diversity. In our study, we selected sites where at least 50 species were recorded and at least 100 person-days were invested in the surveys. We used rarefaction curves only at one site, Los Amigos, to compare the effectiveness of the two main sampling methods (VES and plots). We constructed individual-based rarefaction curves using the program PAST (Palaeontological Statistics), version 1.78 (Hammer et al. 2001).

We also used non-metric Multidimensional Scaling (nMDS) plots to visualize and compare species compositions among amphibian assemblages within the region. We based our nMDS plots on a Bray-Curtis dissimilarity matrix constructed using species presence/absence data (Clarke and Warwick 1994). We used the statistical software Primer-E, version 5.0 (Primer-E, Ltd., Ivybridge, United Kingdom), to conduct these analyses (Clarke and Warwick 1994). Because the number of unique species (i.e., recorded only at one site) might be associated with the number of species recorded at each site, we used a Spearman rank correlation to assess the relationship between the number of unique species and the total of species recorded at each site. We used Mantel tests (1000 randomizations) to evaluate correlations between species composition and geographic distance among sites ($\alpha = 0.05$). We used a Microsoft Excel (Microsoft Corporation, Washington, integrated with USA) spreadsheet **PopTools** (http://www.cse.csiro.au/poptools, downloaded 15 June

TABLE 1. Species of amphibians recorded at nine localities in Madre de Dios region, southeastern Peru. Acronyms: LA = Los Amigos PI = Piedras, CA = Cuzco Amazónico, CC = Cocha Cashu, CS = Centro Sachavacayoc, EA = Eco Amazonia, EI = Explorer's Inn, PA = Pakitza, TC = Tambopata Center. Shaded acronyms (gray background) denote sites outside of the national system of protected areas. Cells: 1 = Presence, 0 = Absence. CC, CS, EI, PA, and TC are located inside protected areas. 'Cons' denotes whether a species was found only in protected areas (Ins), only outside (Out), or in both categories.

				I	Localitie	s				
Taxon	LA	PI	CA	CC	CS	EA	EI	PA	TC	Cor
ANURA										
Aromobatidae										
Allobates conspicuus	1	1	0	1	0	0	0	1	0	Bot
Allobates femoralis ¹	0	1	1	1	Ő	1	1	1	1	Bot
Allobates trilineatus	1	1	1	1	1	1	1	1	1	Bot
Strabomantidae	1	1	1				1	1		Dot
Noblella myrmecoides	1	0	0	0	1	0	1	1	1	Bot
Oreobates quixensis	1	0	0	1	0	1	0	1	1	Bot
Pristimantis altamazonicus	1	1	1	1	1	1	1	1	1	Bot
Pristimantis duccinator	1	1	0	1	0	0	0	0	0	Bot
Pristimantis carvalhoi	1	0	1	1	1	Ő	0	Ő	0 0	Bot
Pristimantis croceoinguinis	0	0	0	0	0	Ő	1	1	1	Ins
Pristimantis cruralis	1	1	1	Ő	1	1	1	1	1	Bot
Pristimantis diadematus	0	0	0	1	1	1	0	1	0	Bot
Pristimantis fenestratus	1	1	1	1	1	1	1	1	1	Bot
Pristimantis jenestratus Pristimantis imitatrix	0	0	1	0	0	0	0	0	1	Bot
Pristimantis initiarita Pristimantis lacrimosus	0	0	0	0	0	0	1	0	1	Bot
Pristimantis mendax	0	0	0	1	0	0	0	0	1	Ins
Pristimantis menaax Pristimantis ockendeni	1	0	1	1	0	0	0	1	1	Bot
Pristimantis ockenaeni Pristimantis olivaceus	0	0	0	0	0	0	0	1	0	Ins
Pristimantis veichlei	1	1	1	1	1	1	1	1	1	Bot
Pristimantis skydmainos	0	1	0	1	1	1	0	0	1	Bot
Pristimantis skyamainos Pristimantis sulcatus	0	0	0	1	0	0	0	0	0	Ins
Pristimantis suicatus Pristimantis tantanti	0	0	0	1	0	0	0	0	0	Ins
	1	1	1	1	1	0	1	1	1	Bot
Pristimantis toftae Pristimantis ventrimarmoratus	1	1	1	1	0	0	0	1	1	Bot
	1	1	1	1	0	0	0	1	1	BOI
Bufonidae	1	0	0	0	1	0	1	0	1	Bot
Dendrophryniscus minutus $Phacha auttatus^2$	1	0	1	1	1	1	1	1	1	Bot
Rhaebo glaberrimus/Rhaebo guttatus ²	1	1	1	0	1	1	1	1	1	Bot
Rhinella margaritifera ³	-	1	1			1			1	
Rhinella marina	1			1	1		1	1		Bot
Rhinella poeppigii	0	0	0	0	0	0	1	0	1	Ins
Centrolenidae	1	0	0	0	0	0	0	1	0	D (
Cochranella midas	1	0	0 0	0	0 0	0	0	1 0	0	Bot
<i>"Centrolenella"</i> sp.	0	0	0	0	0	0	1	0	0	Ins
Ceratophrynidae	1			1	1	1		1	1	D (
Ceratophrys cornuta	1	1	1	1	1	1	1	1	1	Bot
Dendrobatidae				1	1	1				D (
Ameerega hahneli	1	1	1	1	1	1	1	1	1	Bot
Ameerega macero ⁴	0	0	0	0	0	0	0	0	0	Ins
Ameerega picta ⁵	0	0	0	0	0	0	0	0	0	Ins
Ameerega trivittata	1	1	0	1	1	0	1	1	1	Bot
Ranitomeya biolat	1	0	0	1	0	0	1	1	1	Bot
Ranitomeya cf. ventrimaculata	1	0	0	0	0	0	0	0	0	Ou
Hemiphractidae		c	~	<i>c</i>	<i>c</i>	<i>c</i>	c	~	<i>c</i>	~
Hemiphractus helioi	1	0	0	0	0	0	0	0	0	Ou
Hemiphractus scutatus	1	0	0	1	0	0	0	1	1	Bot
Hylidae										
Cruziohyla craspedopus	1	0	1	1	0	0	1	0	1	Bot
Dendropsophus acreanus	1	0	0	0	0	0	1	1	0	Bot
Dendropsophus allenorum	1	0	1	0	0	1	1	0	0	Bot
Dendropsophus bokermanni	0	0	1	0	0	0	1	0	0	Bot
Dendropsophus brevifrons	0	1	1	0	0	0	0	0	0	Ou
Dendropsophus koechlini	1	0	1	1	1	1	1	0	1	Bot
Dendropsophus leali	1	0	1	1	1	1	1	1	1	Bot
Dendropsophus leucophyllatus	1	1	1	1	1	1	1	1	1	Bot
Dendropsophus marmoratus	0	0	1	0	0	0	1	0	0	Bot
Dendropsophus minutus/D. delarivai ⁶	1	1	0	1	0	0	1	0	0	Bot

Table continued on next page.

TABLE 1. continued.

	Localities									
Taxon	LA	PI	CA	CC	CS	EA	EI	PA	TC	Con
Hylidae continued										
Dendropsophus parviceps	1	1	1	1	1	1	1	1	1	Both
Dendropsophus rhodopeplus	1	1	1	1	1	0	1	1	1	Both
Dendropsophus riveroi	0	0	0	1	1	0	1	0	0	Ins
Dendropsophus rossalleni	0	0	0	0	1	0	1	0	1	Ins
Dendropsophus sarayacuensis	1	1	0	1	1	0	1	1	1	Both
Dendropsophus schubarti	1	0	1	1	0	0	0	0	0	Both
Dendropsophus triangulum	1	1	0	1	0	1	0	0	0	Both
Hypsiboas boans	1	1	1	1	1	0	1	1	1	Both
Hypsiboas calcaratus	1	1	1	1	0	1	1	1	1	Both
Hypsiboas cinerascens	1	1	1	1	1	0	1	1	1	Both
Hypsiboas fasciatus	1	1	1	1	1	1	1	1	1	Both
Hypsiboas geographicus	1	0	1	1	0	0	1	0	1	Both
Hypsiboas lanciformis	1	1	0	1	0	0	1	1	1	Both
Hypsiboas punctatus	1	1	1	1	1	1	1	0	1	Both
Osteocephalus buckleyi	1	0	0	0	0	0	0	0	0	Out
Osteocephalus cf. leprieurii	1	1	1	1	1	1	1	1	1	Both
Osteocephalus taurinus	1	1	1	1	1	1	1	0	1	Both
Osteocephalus cf. pearsoni	0	0	0	0	0	0	0	0	1	Ins
Osteocephalus sp. ⁷	1	0	0	1	0	0	0	0	0	Both
Phyllomedusa atelopoides	0	1	1	1	0	0	1	1	0	Both
Phyllomedusa bicolor	1	1	0	0	1	1	1	0	1	Both
Phyllomedusa camba	1	1	1	1	1	0	1	1	1	Both
Phyllomedusa palliata	1	1	1	1	0	0	1	1	1	Both
Phyllomedusa tomopterna	1	1	1	1	1	1	1	1	1	Both
Phyllomedusa vaillanti	1	1	1	1	1	1	1	1	1	Both
Pseudis paradoxa	0	0	1	0	0	0	0	0	0	Out
Scarthyla goinorum	1	1	1	1	1	1	1	1	1	Both
Scinax chiquitanus	0	0	1	0	0	0	1	1	0	Both
Scinax garbei	1	1	1	1	1	1	1	0	1	Both
Scinax ictericus	1	1	1	1	1	1	1	0	1	Both
Scinax pedromedinae	1	1	1	1	1	1	1	1	1	Both
Scinax ruber	1	1	1	1	1	1	1	1	1	Both
Sphaenorhynchus dorisae	0	0	0	1	0	0	0	0	0	Ins
Sphaenorhynchus lacteus	1	1	1	1	0	1	1	0	0	Both
Trachycephalus coriaceus	1	0	1	1	1	1	1	1	1	Both
Trachycephalus resinifictrix	0	0	0	1	0	0	0	0	0	Ins
Trachycephalus venulosus	1	1	1	1	1	1	1	1	0	Both
Leiuperidae										
Edalorhina perezi	1	1	1	1	0	1	0	1	0	Both
Physalaemus freibergi	1	1	1	1	1	1	1	1	1	Botł
Leptodactylidae		_	-	c.						-
Leptodactylus bolivianus	1	0	1	0	1	1	1	1	1	Both
Leptodactylus didymus	1	1	1	1	1	1	1	1	1	Both
Leptodactylus discodactylus	0	0	0	0	1	0	1	0	0	Ins
Leptodactylus knudseni	1	1	1	1	0	0	1	1	1	Both
Leptodactylus leptodactyloides	1	1	1	1	1	1	1	1	1	Both
Leptodactylus lineatus	1	1	1	1	1	1	1	1	1	Both
Leptodactylus pentadactylus	1	1	1	1	1	1	1	1	1	Botl
Leptodactylus petersii	1	1	1	1	1	1	1	1	1	Botl
Leptodactylus rhodomystax	0	1	0	1	1	1	1	1	1	Both
Leptodactylus rhodonotus	0	0	1	1	0	0	1	1	1	Both
Leptodactylus stenodema	1	0	0	0	0	0	0	0	0	Out
Leptodactylus sp. (Adenomera spp.) ⁸	1	1	1	1	1	1	1	1	1	Both
Microhylidae										
Altigius alios	0	0	1	0	0	1	0	0	0	Out
Chiasmocleis bassleri	1	1	0	0	0	0	0	0	1	Both
Chiasmocleis ventrimaculata	1	1	1	1	1	1	1	1	1	Both
Ctenophryne geayi	1	0	1	1	1	1	1	1	1	Both
Elachistocleis bicolor	1	1	1	1	1	1	1	0	1	Both

Table continued on next page.

TABLE 1. continued.

					Lo	calities					
Taxon	LA	PI	CA	CC	CS	EA	EI	PA	TC	Cons	
Microhylidae continued											
Hamptophryne boliviana	1	1	1	1	1	1	1	1	1	Both	
Syncope antenori	1	0	0	0	0	0	0	0	0	Out	
Pipidae											
Pipa pipa	1	0	1	1	0	0	1	0	1	Both	
CAUDATA											
Plethodontidae											
Bolitoglossa altamazonica	1	0	0	1	0	0	0	1	1	Both	
GYMNOPHIONA											
Caeciliidae											
<i>Caecilia</i> sp.	0	0	0	0	0	0	0	0	1	Ins	
Oscaecilia sp. (bassleri in CC)	1	0	0	1	0	0	0	0	0	Both	
Siphonops annulatus	1	0	0	0	0	0	0	0	0	Out	

Notes: (1) Allobates femoralis is present near LA, on the other side of Madre de Dios river. (2) Rhaebo glaberrimus and R. guttatus occupy the same row because previous locality records did not explicitly differentiate these species, however only one of them occurs at any locality (Duellman 2005); R. guttatus has been recorded at LA. (3) Rhinella margaritifera was formerly recognized as Bufo "typhonius" or Bufo margaritifer; it represents more than one species. (4) Ameerega macero is present near CC, in the terra firme forest across the Manu river (Rodríguez and Myers 1993) but not in any of the nine localities. (5) A. picta has been recorded at Lago Sandoval (Duellman 2005) but not in any of the nine localities. (6) Dendropsophus minutus and D. delarivai occupy the same row because previous locality records did not explicitly differentiate these species, however in is likely that only one of them occurs at any locality; D. delarivai has been recorded at CC, whereas D. minutus has been recorded at LA. (7) New, undescribed Osteocephalus species (Catenazzi and Rodríguez, pers. comm.). (8) Formerly Adenomera sp., four species have been recognized in Madre de Dios (Angulo et al. 2003) but identification in the field is difficult.

2007) to perform Mantel tests.

RESULTS

Lowland amphibian fauna of Madre de Dios.-The lowland rainforests of Madre de Dios region contain 114 recognized species of amphibians in 38 genera (Table 1). This reflects a compilation of our work at Los Amigos and Las Piedras and inventories from seven other sites Twenty-six species (22.8%) are (Duellman 2005). present in all nine lowland sites, whereas 15 species (13.2%) occur at a single site. Los Amigos is the most species-rich locality in the region with 82 species of amphibians in 34 genera, and accounts for 72% of the amphibians recorded to date in Madre de Dios (Table 2). Our observed species count underestimates the actual species richness because several *Leptodactylus* (formerly Adenomera sp.) species remain unidentified and so we refer to them as Leptodactylus sp. in our species list (Table 1). Four species of *Leptodactvlus* sp. (formerly Adenomera sp.) occur in Madre de Dios (Angulo et al. 2003). Thus, one Leptodactylus sp. is listed for all sites although it is actually a composite of several unidentified species. Furthermore, low species richness at some localities may result from lower sampling effort at those sites (Table 2; see also Discussion).

Our species list includes several additions and corrections to previously published lists (Doan and Arizabal Arriaga 2002; Duellman 2005). We added *Ameerega macero* to our species list because this

species' description originated from Manu National Park (Rodríguez and Myers 1993), though it was not included in the list of amphibians of Madre de Dios (Duellman 2005). Even though A. macero was originally reported from Cocha Cashu (Rodríguez and Cadle 1990), we did not include it in the species records for Cocha Cashu (Table 1) because it was found on the other side of the Manu river (Rodríguez and Myers 1993). This criterion of excluding a species record of a particular site if the species was recorded only on the other side of a major river was applied by previous researchers (e.g., Duellman 2005). Pristimantis tantanti is a new species (Lehr et al. 2007), previously referred to as Pristimantis acuminatus in Cocha Cashu (L. Rodríguez, personal observation). Pristimantis reichlei is another new species (Padial and De la Riva 2009), previously referred to as Pristimantis peruvianus in Madre de Dios. Physalaemus freibergi is also a new species (Funk et al. 2008), previously referred to as *Physalaemus petersi* in Madre de Dios. Dendropsophus delarivai is a relatively new species (Köhler and Lötters 2001), which was previously confused with Dendropsophus minutus in Cocha Cashu (L. Rodríguez, personal observation). Six species from Los Amigos represent new records for Madre de Dios. Three of these species, Leptodactylus stenodema, Osteocephalus buckleyi, and Syncope antenori are now known from Los Amigos (von May 2004a, 2004b; von May et al. 2007). We provide the first report of Ranitomeva cf. ventrimaculata and Hemiphractus helioi in Madre de Dios (Table 1). The

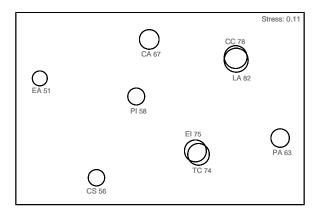


FIGURE 2. Non-metric Multidimensional Scaling plot depicting differences in amphibian species composition among nine lowland sites in Madre de Dios, Peru. Plot is based on presence-absence data and Bray-Curtis dissimilarity measure. Relative position of symbols indicates the degree of similarity between sites; size of circles is proportional to the number of species recorded at each site. See Fig. 1 for site abbreviations.

sixth species, *Osteocephalus* sp., is a previously undescribed species and also occurs in Cocha Cashu (A. Catenazzi and L.O. Rodríguez, unpubl. data).

Although the sampling effort differed among sites, the similarity matrix (Table 3) provides an overview of the relationship among amphibian assemblages. Los Amigos is most similar to Cocha Cashu (Table 3), and they share 66 species (CBR = 0.825); these sites cluster together in the nMDS plot (Fig. 2). Los Amigos and the Tambopata Center share several species (CBR = 0.795), but this site clusters with the Explorer's Inn site (Fig. 2). Las Piedras shares species with Cocha Cachu (CBR = (0.779) and Los Amigos (CBR = 0.757), but this site did not cluster with either Los Amigos or Cocha Cashu in the nMDS plot (Fig. 2). The number of unique species we detected is strongly associated to the number of species known to be present at each site (Spearman rank correlation, r = 0.932, P < 0.001). We found no correlation between species composition and geographic distance among sites (Mantel test, r = 0.325, P = 0.054). This result suggests that spatial autocorrelation probably

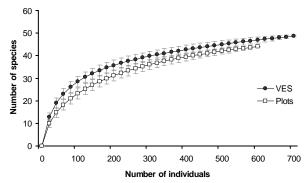


FIGURE 3. Rarefaction curves based on data collected using visual encounter surveys (VES) and leaf-litter plots (Plots) at Los Amigos, Peru. Each curve represents the expected number of species for a given number of observed individuals. The bars indicate \pm standard deviation.

has a minimal effect on species composition.

We evaluated the potential effect of sampling effort on observed species richness using rarefaction curves based on data from Los Amigos. We used this dataset because it was the only one for which we had multi-year samples, as data from other sites were not available and data from Las Piedras included only two sampling periods. We observed that the accumulation curves for Los Amigos approached the asymptote, suggesting that most species that could theoretically be detected with VES and plots were actually detected at Los Amigos (Fig. 3). However, because of the relatively high number of singletons (12 singletons in VES and five singletons in plots), more species could be expected with both Overall, the observed trends sampling methods. indicated that VES were slightly more effective in detecting additional species (Fig. 3). We recorded 49 amphibian species using VES (2003-2007), 44 species using plots (2003 and 2007), and 58 species combining both methods. The remaining species records at Los Amigos were obtained through opportunistic sampling.

TABLE 2. Nine lowland sites in Madre de Dios, Peru with inventoried amphibian faunas, number of person-days invested in the inventories, and number of species recorded. See Table 1 for site abbreviations and complete species lists. Shaded abbreviations (gray background) denote sites outside of the national system of protected areas (see Fig. 1).

Abbreviation	Locality	Total effort [*] (person-days)	Total amphibia [*]	Anura / Apoda / Caudata
CA	Cuzco Amazónico	1473 ^{bc}	67	67 / 0 / 0
CC	Cocha Cashu	395 ^d	78	72 / 1 / 1
CS	Centro Sachavacayoc	348 ^c	56	56 / 0 / 0
EA	Eco Amazonia	380 ^c	51	51 / 0 / 0
EI	Explorer's Inn	380 ^c	75	75 / 0 / 0
LA	Los Amigos	711 ^a	82	79 / 2 / 1
PA	Pakitza	286 ^e	63	62 / 0 / 1
PI	Las Piedras	102 ^a	58	58 / 0 / 0
TC	Tambopata Center	336 ^c	74	72 / 1 / 1

*Note: Sources for total effort and number of species: (a) This study, (b) Duellman 2005 (1081 pers.-days) and (c) Doan and Arizabal Arriaga 2002 (392 pers.-days in CA), (d) Rodríguez and Cadle 1990, Rodríguez 1992, (e) Morales and McDiarmid 1996.

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TABLE 3. Number of amphibian species recorded at nine sites in Madre de Dios region, southeastern Peru. The number of species at each site is highlighted in boldface in the diagonal. The number of species common to the two sites is above the diagonal (upper right). The coefficient of biogeographic resemblance is in italics below the diagonal (lower left). See Table 1 for site abbreviations and complete species lists. Shaded abbreviations (gray background) denote sites outside of the national system of protected areas.

	CA	СС	CS	EA	EI	LA	PA	Ы	тс
CA	67	56	45	45	58	57	48	47	54
CC	0.772	78	48	45	58	66	54	53	60
CS	0.732	0.716	56	43	53	50	43	43	51
EA	0.763	0.698	0.804	51	45	46	39	41	44
EI	0.817	0.758	0.809	0.714	75	61	53	50	63
LA	0.765	0.825	0.725	0.692	0.777	82	55	53	62
PA	0.738	0.766	0.723	0.684	0.768	0.759	63	45	54
PI	0.752	0.779	0.754	0.752	0.752	0.757	0.744	58	49
TC	0.766	0.789	0.785	0.704	0.846	0.795	0.788	0.742	74

We also used an nMDS plot to visualize the relationship between species composition and forest types at Los Amigos (Fig. 4). Based on species presence/absence data, each forest type formed a separate cluster, suggesting that the amphibian assemblages differed among habitats across five years of sampling. We found that the floodplain had the highest number of species (38 species) compared to the other forest types (Table 4). The other three forest types had very similar species richness, with 14-16 species less than the floodplain (Table 4). We recorded some species in only one forest type using VES. The floodplain, with 11 species, had more 'unique' species (i.e., recorded in one habitat only) than the other habitats. The terra firme and bamboo both had four 'unique' species, and the palm swamp had three 'unique' species.

Amphibian fauna recorded inside and outside protected areas.—We recorded 105 species in protected

areas of Madre de Dios and 96 species in unprotected areas (Table 1). Seventeen species occurred exclusively inside areas that belong to the national protected area system (SINANPE) and nine species occurred exclusively in sites that do not belong to this system The number of amphibian species we (Table 1). recorded inside preserves varied greatly and there was no relationship between species richness and size of the preserve (Table 5). For example, the Tambopata National Reserve and Manu National Park have approximately the same number of species even though the Tambopata preserve is six times smaller than Manu National Park. We only included species recorded in the lowlands of Manu (< 500 m) in this comparison. Many other species in Manu inhabit montane forests and Andean grasslands and occur in the Cusco region. Manu and Tambopata each contain about 81% of the amphibians recorded in Madre de Dios. Despite these

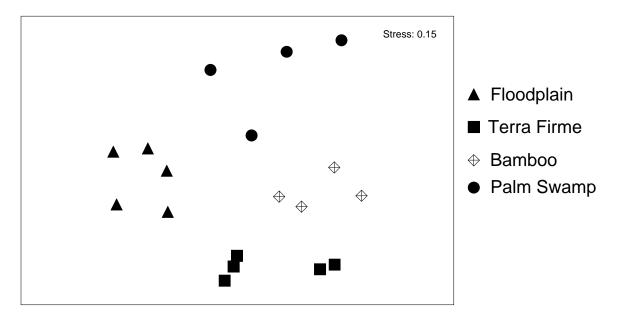


FIGURE 4. Non-metric Multidimensional Scaling plot depicting the relationship between species composition and forest types at Los Amigos station, Peru. Based on presence/absence data collected with visual encounter surveys during five sampling periods.

TABLE 4. Number of amphibian species in four forest types at Los Amigos Research Center, Peru. The data were recorded with nocturnal VES (50×4 m) between 2003 and 2008. The number of species in each habitat is highlighted in boldface in the diagonal. The number of species common to each pair of habitats is below the diagonal (lower left).

	Floodplain	Terra firma	Bamboo	Palm swamp
Floodplain	38			
Terra firma	13	23		
Bamboo	15	10	24	
Palm swamp	15	9	11	22

differences, all preserves combined had 105 species of amphibians, or 92% of the amphibians in Madre de Dios.

DISCUSSION

Lowland amphibian fauna of Madre de Dios.-At least 114 species of amphibians, represented by 110 anurans, three caecilians, and one salamander currently reside in Madre de Dios region (Table 1). We found that four species (Rhinella marina, Rhinella margaritifera, Leptodactylus sp., and Scinax ruber) out of the 26 species that occur in all lowland sites of Madre de Dios are also common and widespread in most of Brazilian Amazonia (Azevedo-Ramos and Galatti 2002). The Los Amigos Research Center is the most species-rich locality in Madre de Dios. Though many previous inventories took place at the lowland sites included in this study, the rate of species accumulation in some of them (e.g., Eco Amazonia, Centro Sachavacayoc) suggests that components of this region's amphibian diversity remain unidentified (Doan and Arizabal Arriaga 2002). It is likely that more intensive sampling will contribute between one to 10 additional species at some sites, particularly the sites that exhibit the lowest species richness. This assumption follows that nine additional species were recorded at one site (Cuzco Amazónico) by Duellman (2005), compared to the previous list presented for that same site (Doan and Arizabal Arriaga 2002).

Most comparisons among sites in Madre de Dios had relatively high CBR values, slightly higher than those previously recorded in Madre de Dios (Duellman 2005). We found a relatively large similarity in species composition among sites (51.03 ± 6.80 [SD] species are in common for pairs of sites, N = 36). The CBR values we recorded are greater than average CBR values recorded from other rainforest and savanna regions in South America (Azevedo-Ramos and Galatti 2002, Donnelly et al. 2005b). Most of the comparisons using CBR values in Azevedo-Ramos and Galatti (2002) and Donnelly et al. (2005b) involved large geographic scales, resulting in relatively small CBR values. However, comparisons among forest sites located only in Guyana (with a geographic scale comparable to

Madre de Dios) gave CBR values that were smaller than the values we saw in Madre de Dios (Donnelly et al. 2005a, 2005b; Duellman 2005). It was suggested that forests in Madre de Dios are more homogenous than those in Guyana (Donnelly et al. 2005b), and thus resulted in high similarity of species composition among sites. The high CBR values observed in Madre de Dios may result from pooling species records from different habitats into a single species list for each site.

Geographic distances observed in our analyses did not explain the variation in species composition observed among sites in Madre de Dios. Previous studies had similar results (Doan and Arizabal Arriaga 2002), although they included only the five Tambopata sites (CA, CA, EA, EI, TC; Fig. 1). In contrast, two studies that included more sites and encompassed a larger geographic scale than our study indicated that there is a significant negative correlation between species similarity and geographic distance among sites (Azevedo-Ramos and Galatti 2002; Duellman 2005). Both studies included inventory data that also differed in terms of sampling effort, study period, number of researchers, and sampling methodology. Since data from large-scale standardized inventories with multiple site comparisons are rare, researchers in Brazil combined data from different inventories to provide an overview of the amphibian diversity in Amazonian regions (Azevedo-Ramos and Galatti 2002).

Several biotic and abiotic factors (different than geographic distance) may affect the observed patterns of species composition and similarity between sites. For example, researchers have found that soil clay content, leaf litter depth, and tree density may affect the distribution of particular species or groups of species in tropical forests (e.g., Lieberman 1986; Fauth et al. 1989; Ernst and Rödel 2005; Watling 2005; Menin et al. 2007). It is plausible that some of these factors vary among the nine studied sites in Madre de Dios, thus affecting the presence/absence of particular amphibian species.

 TABLE 5.
 Natural protected areas in Madre de Dios region, Peru and amphibian species richness.

Natural protected area	Area ^a (ha)	Amphibian species
Amarakaeri Communal Reserve	402,336	2 ^b
Manu National Park	1,696,803	91
Bahuaja-Sonene National Park	1,091,416	43
Tambopata National Reserve	274,691	92

Sources: (a) Amazon Conservation Association GIS office; (b) only *Rhinella marina* and *Rhinella margaritifera* have been recorded in Amarakaeri (Valdés-Velásquez, A. 2005. Informe Final: Estudio del Potencial Biológico de la Reserva Comunal Amarakaeri y Tierras Indígenas Aledañas. Proyecto "Conservación y Uso Sostenible de la Biodiversidad en la Reserva Comunal Amarakaeri y Tierras Indígenas Aledañas". GEF/PNUD-FENAMAD. Unpublished report, Lima. Peru).

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As hypothesized by Rodríguez and Cadle (1990), our findings suggest that habitat heterogeneity influences the patterns of amphibian species richness and composition at local (i.e., one site in Madre de Dios) and regional (all of Madre de Dios) scales. Our sampling at Los Amigos, a site that contains all major terrestrial habitats and aquatic habitats, indicated that there is a relationship between amphibian species composition and forest types (Fig. 4). If amphibians are segregating by habitat (forest type) on a local scale, then extrapolating to a regional scale would theoretically justify the importance of preserving heterogeneous landscapes in Madre de Dios. More studies on the connection between habitat heterogeneity and amphibian diversity patterns are needed to test this idea. Information on the relationship between animal assemblages and the habitats they use, implemented with other taxonomic inventories, will allow us to economize resources via maximizing the species diversity protected when designing nature preserves (Silvano and Segalla 2005).

We think that our comparison of species lists provides a thorough summary of amphibian species composition among sites in the Madre de Dios region. We are aware that the species lists for some sites used in this study may not have been fully completed at the time of our assessment, but other sites, like Los Amigos, were thoroughly sampled. At Los Amigos, we observed that the rarefaction curves approached the asymptote suggesting that most species were recorded using two standard methods. Because many species records at Los Amigos (as well as in other sites) were obtained through opportunistic sampling, it is difficult to compare detectability across sites. We think that our ability to detect a species was consistent across habitats at Los Amigos because we used the same procedures and personnel for all sampling. We feel confident that nearly six years of data from our main study site (Los Amigos) provided sufficient information that allows us to compare observed amphibian community composition across forest types.

Overall, we think that the inclusion of two new sites (Los Amigos and Las Piedras) has improved our current understanding of Madre de Dios's amphibian diversity. Other researchers adopted this approach in previous studies (e.g., Azevedo-Ramos and Galatti 2002; Donnelly et al. 2005b) and improved the knowledge of other regions' amphibian diversity.

The relationship between amphibian diversity and protected areas.—Protected areas contain 91% of all amphibian species found in Madre de Dios and most amphibian species are in Tambopata National Reserve and Manu National Park (Table 5). In contrast, only two species occur in the Amarakaeri Communal Reserve. However, the records from Amarakaeri were incidental because no formal inventory of this preserve exists. We of protected areas, Altigius alios, was considered Data

did not find a relationship between similarity and geographic distance. This makes comparing amphibian assemblages at Amarakaeri Communal Reserve to those at Los Amigos and the Manu sites (i.e., Cocha Cashu and Pakitza), or to the Tambopata sites (e.g., Explorer's Inn, Tambopata Center), difficult. We also expect to record more species within the Los Amigos watershed because part of the watershed is closer to the Manu sites (CC and PA), which have some species not recorded in other sites (e.g., LA). Because conservation of biological diversity in southern Peru faces many threats (i.e., large-scale agriculture, logging, and cattle ranges), we think that the establishment of a corridor between the northern and southern preserves of Madre de Dios is a priority for ensuring the long-term functioning of the region's ecosystems (Thieme et al. 2007).

Los Amigos and Cuzco Amazónico are important for the conservation of the nine amphibian species found solely outside of nationally-recognized preserves in Madre de Dios. Six of these species were only recorded at Los Amigos while three were only recorded at Cuzco Amazónico (Table 1). In this context, both sites are complementary to the other protected areas in Madre de Dios in terms of conservation of habitat for these species. Even though these two sites do not belong to the national system of preserves (SINANPE), they are protected by the type of land use: Los Amigos is a conservation concession managed by a non-profit organization (Amazon Conservation Association) and Cuzco Amazónico is a private ecotourism reserve. The Los Amigos Conservation Concession was created to protect the biodiversity in the region and its goals are similar to those of an official protected area. The difference is that Los Amigos is managed by a non-profit organization, which obtained a 40-year concession to help the Peruvian government protect and manage 145,918 ha of lowland forest (Pitman 2006). Similarly, private ecotourism reserves like Cuzco Amazónico and ecotourism concessions like Las Piedras effectively complement the goals of nationally-recognized protected areas in Peru. However, all conservation lands termed "concessions" by the Peruvian government are at risk of being changed into a variety of resource-extraction concessions.

Conservation Status.-We recorded several species of amphibians that are included in the IUCN Red List (IUCN, Available from http://www.iucnredlist.org, [Accessed 15 August 2007]). The anurans Pristimantis olivaceus and Allobates conspicuus were classified as Data Deficient and both have been recorded inside and outside the protected area system in Madre de Dios. These species occur in the lowlands (< 500 m elevation) of Manu National Park (A. conspicuus also occurs at Los Amigos). One of the nine species recorded only outside

Deficient by the IUCN and was considered Near Threatened by the Peruvian government (INRENA. 2004. Categorización de especies amenazadas de fauna Silvestre. Aprobado por Decreto Supremo N° 034-2004-AG. El Peruano Available from <u>http://www.inrena.gob.pe</u>, [Accessed 15 August 2007]). The other eight species were classified as Least Concern by the IUCN, and were not evaluated by the Peruvian government.

We did not include 28 threatened amphibian species that occur in the Madre de Dios river basin in our species list, because those species occur outside the political boundary of Madre de Dios region. However, we mention them here because most of those species occur in at least one large preserve in Madre de Dios whose boundary also extends into another region (e.g., Manu National Park includes lands in Madre de Dios and Cusco regions). These species have restricted distributions and inhabit the montane forests of Cusco, near Manu National Park; as well as, the montane forests of Quincemil and Sandia Provinces in the north of Puno department. Even though those particular species were not recorded in Madre de Dios, the fact that they occur inside a preserve is important for the assessment of their conservation status. For example, Phrynopus cophites and Phrynopus peruvianus inhabit montane forests of Manu National Park in Cusco region and have been classified as Endangered by the IUCN Red List. Ameerega simulans, a species found in Bahuaja-Sonene National Park, was the only species listed as Endangered by the Peruvian government (INRENA. 2004. op. cit.) and currently faces a critical situation caused by policy changes favoring oil exploration inside National Parks. We did not include these species in our species list, because these species were recorded in Puno and Cusco regions but not vet in Madre de Dios region (Mvers et al. 1998; Duellman 2005).

We recorded four species of amphibians at Los Amigos that were classified by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (CITES. Available from <u>http://www.cites.org</u> [Accessed 15 August 2007]): Allobates femoralis, Ameerega trivittata, Ranitomeya biolat, and Ranitomeya cf. ventrimaculata (the former generic names Epipedobates and Dendrobates, for Ameerega and Ranitomeya, are respectively listed). The CITES Appendix II includes species that are not currently threatened with extinction, but which may become threatened in the future because of uncontrolled pet trade (CITES. op. cit.).

Although current IUCN and CITES criteria list most lowland amphibians in the Least Concern category, it is important to note that lowland forests are not safe from amphibian population declines. There is evidence that lowland amphibians have experienced long-term declines in at least one lowland Central American forest

(Whitfield et al. 2007). Even though the potential causes of the declines remain unclear, we believe that the same phenomenon could potentially affect lowland amphibians in South America. We consider that current IUCN Red List criteria can be misleading as no longterm data are available for most lowland Amazonian species. This lack of data does not imply that amphibian populations in lowland Amazonia are impervious to factors (e.g., chytridiomycosis) causing declines in other regions.

Conclusions.—Our study illustrates the importance of non-protected areas for amphibians in Madre de Dios region, particularly in terms of their contribution to local and regional biodiversity. We found that areas that belong to the national system of protected areas (SINANPE) include more than 90% of the amphibian species recorded in the region and that areas outside of the national system additionally contained a large number of species, some of which were not found in protected areas. Thus, it is important that an increasing number of non-protected sites are inventoried as they will provide additional information for the future creation of nationally-recognized preserves. In particular, these inventories should be conducted in sites located between the northern and southern preserves of Madre de Dios in order to fill a large gap of nonprotected lands. Ideally, any new preserve should be large enough to include all major forest types and aquatic habitats in order maximize the representation of the regional diversity. Based on the data produced by our study, all forms of preserves, ranging from national parks to biological stations to private ecotourism lodges, will be effective and necessary for protecting wildlife in Madre de Dios.

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