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ORIGINAL ARTICLE

Natural re-establishment of a population of a critically endangered primate in a secondary forest: the San Martin titi monkey (*Plecturocebus oenanthe*) at the Pucunucho Private Conservation Area, Peru

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Abstract The San Martin titi monkey (*Plecturocebus oenanthe*) is endemic to a small area of northern Peru and is considered Critically Endangered on the IUCN due to massive habitat loss. Between 1994 and 2005 small scale reforestation efforts in the 23.5 ha area of Pucunucho have led to the recuperation of habitat from an area of pasture and crop lands. The first record of *P. oenanthe* re-establishment in the area is from 2010, although re-establishment probably began earlier. We carried out short population surveys using triangulation to monitor densities of *P. oenanthe* in Pucunucho in 2011, 2012 and 2016. We estimate the current population of *P. oenanthe* in this area at 27 individuals, giving population densities of 35 groups/km^2 and 124 individuals/km^2. The successful regeneration of habitat and natural re-population of the area by this Critically Endangered species provides evidence of successful reforestation based conservation activities for this and potentially other primate species. Although now protected as a Private Conservation Area, Pucunucho remains threatened.

Keywords Reforestation · Enrichment planting · Triangulation · Density · Conservation

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Introduction

Globally, habitat fragmentation is a primary cause of increased extinction risk for primates and many other species (Arroyo-Rodríguez and Mandujano 2009; Marsh et al. 2013; Benchimol and Peres 2014). The region of San Martin in northern Peru has one of the highest levels of deforestation in the country (Llactayo et al. 2013), and subsequently high levels of habitat fragmentation. In the 1980s the construction of the Marginal Highway and increasing coca (*Erythroxylum coca*) cultivation resulted in mass immigration and associated deforestation in San Martin (Young 1996; Fjeldså et al. 2005; Shanee 2011). The current high levels of deforestation in San Martin region is driven by sustained immigration into the region and subsequent conversion of forests to rice paddies, pasture and for subsistence crops, as well as demand for timber, other cash crops (Coffee and cacao) and other forest resources (Shanee 2012).

The San Martin titi monkey (*Plecturocebus oenanthe*: Thomas 1924, Sensu Byrne et al. 2016) is one of Peru's 8 endemic primate species (Matauschek et al. 2011; Wilson et al. 2013; Aquino et al. 2015). It is restricted to the north of San Martin region in areas below 1200 m above sea level (m.a.s.l.) west of the Rio Huallaga and north of the Rio Huayabamba (Bóveda-Penalba et al. 2009; Shanee et al.

2011). This species is considered Critically Endangered by the IUCN (2011) and under Peruvian law (D.S. 004-2014-MINAGRI); it is also on the list of the "Top 25 Most Threatened Primate Species" (Schwitzer et al. 2016). This species' highly threatened status is a result of the extremely high levels of habitat loss and fragmentation throughout its restricted range (Bóveda-Penalba et al. 2009; Shanee et al. 2011). Variable population densities have been reported for this species. Aldrich et al. (2008) reported densities of 1.41 individuals/ha at Tarengue, a large fragment of disturbed primary and regenerating secondary forest. van Kuijk et al. (2015) carried out a similar study in the Ojos de Agua Conservation Concession, reporting densities of 4 groups/km^2 for forest interior and 16.5 groups/km^2 for forest edge. Both studies employed triangulation for their surveys.

Secondary forests are often associated with higher primary production levels than primary forests (Lugo and Frangi 1993) and can provide habitat for primates, particularly small bodied species, and other animals (Parry et al. 2007). Enrichment planting, the aiding of natural regeneration through planting of key forest species within secondary regrowth (Parrotta et al. 1997) accelerates habitat recuperation by planting saplings and by attracting seed dispersers such as primates, whose activities accelerate regeneration (Parrotta et al. 1997; Vulinec et al. 2006; Shono et al. 2007). Results of previous studies on *P. oenanthe* have suggested a preference for secondary, disturbed and edge forest areas (Mark 2003; Bóveda-Penalba et al. 2009; van Kuijk et al. 2015). Thus, enrichment planting and reforestation may increase suitable habitat for *P. oenanthe*.

Census methods allow the estimation of a species population density in a determined area, with repeat censuses providing measures of changes in these densities on a temporal scale. We used auditory censuses to determine if *P. oenanthe* was able to inhabit regenerating forests, and if so, how this population grew over time. Auditory methods are suggested to be better suited to population surveys of cryptic and vocal species, such as titi monkeys, than traditional line transect methods that rely on visual detection events (Davies 2002). Auditory methods are particularly useful for species that vocalize at predictable times, such as *P. oenanthe* (Aldrich et al. 2008).

In this study we present observations on the aided regeneration of a patch of forest and natural re-establishment of a population of *P. oenanthe* in the south of its range and report results of short surveys using vocalizations to estimate population densities of *P. oenanthe* in a small private conservation area in central San Martin, Peru. We compare our results with those from studies at primary forest sites with less habitat disturbance to improve basic ecological knowledge of this species and better inform application of conservation decisions, particularly focused on habitat regeneration.

Methods

Study site

The 23.5 ha Pucunucho Private Conservation Area (ACP) (76°45′5.828″W, 7°13′24.362″S) is owned and administered by Peruvian NGO 'Amazónicos por la Amazonía' (AMPA). Habitat in the area is characterized as Humid Tropical Forest, below 1100 m.a.sl. and Lower Montane Tropical Forest (bh—MBT) (Holdridge 1967). The majority of the area is 22 year old secondary regrowth, with a small area of older *Aguajal* (*Mauritia flexuosa*) forest, along the west bank of the *Rio Huayabamba* in the province of Mariscal Cáceres in north eastern Peru (Fig. 1). The area is traversed from south east to north east by a small stream, *Quebrada Mangapaquina*, and it is bordered to the north east by another stream, *Quebrada Pucunucho*. Habitat has been recovered through enrichment planting of native tree species over a period of 20 years. The area is surrounded on all sides by agricultural land and pasture, although it retains some connectivity with outlying forest through remnant patches and gallery forests. Annual average temperature for 2011, '12 and '16 was 27.6, 26.6 and 27.3 °C, and annual rainfall was 983.8, 756.3 and 705.2 mm for these years, respectively (data from SENAMHI meteorological station 000379 (Pachiza), retrieved at http://www.senamhi.gob.pe).

Habitat recuperation

K. Pinasco purchased the Pucunucho ACP in 1994, it was an area of cattle pasture and mixed crop with a small riverine area of natural regenerating forest in the south of the area characterized by the presence of *Myroxylon balsamum*, *Ficus* spp., *Spondia mombin* and *Brosimum* sp. Between 1994 and 1996 K. Pinasco began small scale enrichment planting to aid natural regeneration, planting 50–100 seedlings per year. Several native fruiting tree species were planted, mainly *Inga* sp., *Matisia cordata* and *Mauritia flexuosa*, and additional species were planted in a random manner, including *Colubrina* sp., *Vitex seudofolia*, *Myroxylon* sp., *Schizolobium* sp., *Spondias mombin* and *Ficus macbridei*. Further enrichment planting occurred between 2002 and 2005, with the addition of native timber species including *Ocotea* sp., *Swietenia macrophylla* and *Guazuma* sp.

Population survey

We mapped the area with GPS and divided it into a 50 × 50 m grid in ArcGIS (ESRI 2012). Four georeferenced observation points were established within the area, one in the approximate center, with the remaining three

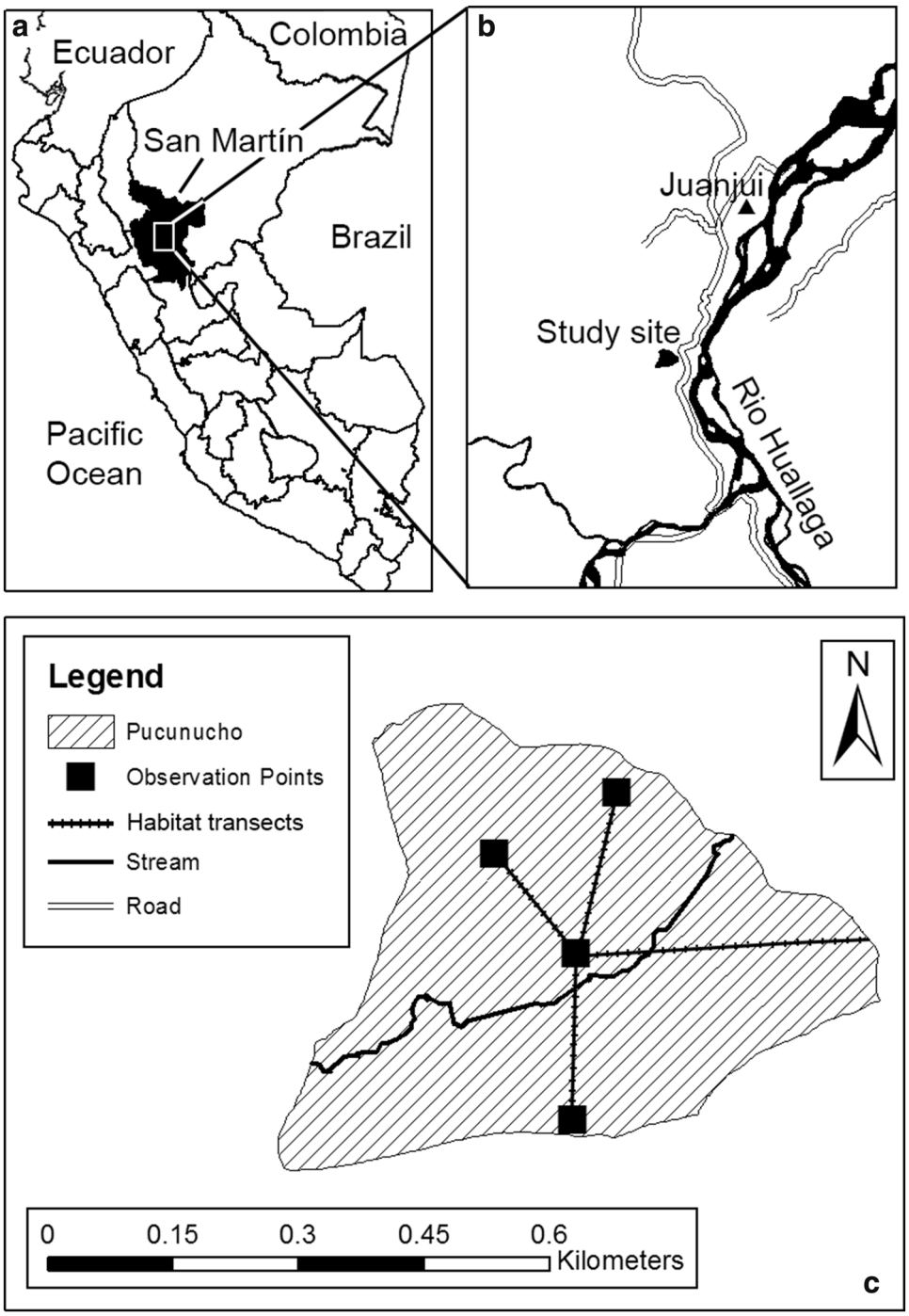

Fig. 1 Map of study area location; *inset* **a** showing location of San Martin department within Peru, *inset* **b** showing location of Pucunucho with major surrounding landmarks and **c** map of Pucunucho showing observation points and transects used in this study

near the limits of the area (Fig. 1). The outer observation points were not equidistant from the central point due to local topography. Observation point locations were selected to increase coverage across the area and avoid physical barriers which potentially may have prevented detecting vocalizations during triangulation. All observation points were between 100 and 200 m apart; this distance was chosen to be well within the critical hearing distance for *P. moloch* of 250 m given by Robinson (1981).

Observations were carried out over 5 days in March 2011, 5 days in October 2012 and 5 days in May 2016. All four observation points were surveyed simultaneously each day between 6:30 and 9:30 am, or until the last vocalization was heard (Aldrich et al. 2008). Each observer recorded the compass bearing and estimated distance to each vocalization from their observation point. Observers also recorded the start and end time of each vocal bout in order to differentiate between calls. We recorded *P. oenanthe* group

size and composition opportunistically upon visual encounters.

In our preliminary analysis, we compared the number of groups recorded in the study area between years using Chi square tests. To calculate the number of groups in the study area during each period, we used the compass bearings of vocalizations recorded by multiple observers to triangulate the location on a map. We also noted the duration of each vocalization to avoid possible confusion with overlapping calls from nearby groups. The locations of visually detected groups which did not correspond with a vocalization event were recorded directly onto the maps. Calls that were estimated to fall outside of the study area were not included in analysis. To minimize possible overestimation of densities we estimated a possible travel distance of 150 m over a 5 min period based on maximum daily travel distance of 1400 m for *Cheracebus torquatus* (Kinsey 1981); allowing us to distinguish calls from the same group in two distinct locations based on time and distance between vocalization events.

The small size and isolation of the study area allowed us to survey the entire area. We were able to estimate the percentage or proportion of groups expected to vocalize during field observation days as a function of survey days and frequency of vocalizations (Brockleman and Ali 1987; Aldrich et al. 2008) using the following formula:

$$\hat{p}(m) = 1 - [1 - p(1)]^m$$

where: $\hat{p}(m)$ is the proportion of groups expected to vocalize over (m) days; p was fixed at 66%, the lowest frequency of daily vocalizations from a published study on titi monkeys (Kinzey et al. 1977).

Habitat description

During the 2016 survey we made a rapid habitat description using the adapted point-quarter method (Krebs 1999). We used four transects to survey the area locating the first point of three transects at each observation point, with a fourth start point on the border of Pucunucho due east of the center listening point (Fig. 1). We sampled every 10 m along each transect, establishing four quadrants at each point (North east, south east, south west and north west). Although 10 m is less than the recommended distance between points the denser secondary forest at Pucunucho meant that even at this short distance we did not sample the same tree in two points successively (Krebs 1999). We also established a radial cut-off distance around each point of 15 m, not sampling trees at greater distances. The use of these distances meant that re-sampling of trees in neighboring points could occur and so we previously decided to discard any trees already sampled from previous points, although in the end this was not necessary. In each quadrant we measured the distance from the point to the nearest tree and a second measurement from the first tree to its nearest neighbor, thus giving us eight sample trees per point. As this area is young regenerating forest we included all trees of CBH (circumference at breast height, 150 cm) >20 cm. For each tree sampled we recorded trunk diameter at breast height, tree height and crown spread. We did not identify the tree species sampled but did note the trees of the genus *Cercropia,* as characteristic of secondary forest, and Palmaceae, as characteristic of the original *Aguajal* forest of the area.

Results

Habitat recuperation

Between the first enrichment planting (1994–1996) and 2002 a covering of secondary forest and early successional *Purma* was established, allowing the enrichment planting of shade tolerant hardwoods between 2002 and 2005. In 1994 the two streams present in the area (*Quebrada Mangapaquina* and *Quebrada Pucunucho*) were seasonal, only flowing during rainy periods. By 2005 both streams became permanent. The whole area regenerated rapidly until 2010 when a large strip was illegally clear-cut for the passage of high tension electricity cables by state power agency *Electro Oriente.* This clear cutting divided the area in two fragments divided by a 30 m strip of deforested land and reduced habitat in the area by 1.4 ha or 6%. During surveys in 2010 to quantify the damage caused by *Electro Oriente* the first observations that *P. oenanthe* had re-established in the area were made. Several other mammal species were also observed to have re-established in the area, including another endemic primate species, the Andean saddle-back Tamarin (*Leontocebus leucogenys*).

Population survey

Due to inclement climatic conditions, of the possible 15 days of field work we collected data on 9 days (3 days per survey period). In total we recorded 703 vocalizations of which 386 were estimated to be within Pucunucho. We discarded vocalization events which were detected by only one observer or were felt to be open to error because of very short vocal period. Detections used in analyses came from 286 vocalization events and 30 visual observations. Fifty-four vocalization events used in analyses were recorded by only one observer but were considered for analysis as they occurred at distances of less than 50 m and over extended time periods (over 40 s). In some cases events detected by only one observer were grouped as a single observation based on time and direction of the detection.

Table 1 Overall results of *P. oenanthe* triangulation surveys at Pucunucho

Survey	Survey detections	Gp/Ha	Gp/km^2	Ind/Ha	Ind/km^2	Gp's in area	Ind in area
April 2011	6 (±0.7)	0.27	27	1.08	108	6.0	23.8
October 2012	8 (±2.1)	0.36	36	1.26	126	8.0	27.8
May 2016	9 (±0.7)	0.41	41	1.38	138	9.1	30.5
Overall	8 (±0.7)	0.35	35	1.24	124	7.7	27.4

From 30 visual encounters we counted 106 animals, including repeat observations of the same groups/individuals. Group size, excluding 2 detections of single individuals, was between two and five individuals, average 3.56 (±0.89). Although there was a small increasing trend in results between years (Table 1; Fig. 2), differences were not significant ($\chi^2 = 0.616$, $df = 2$, $p = 0.734$). Estimates of groups registered per day showed little variation, giving additional confidence in the accuracy of the results. We observed between 6 and 10 groups of *P. oenanthe* (Table 1) each survey day, with a probable total of 8 groups within the study area, giving an estimated population size of 27 individuals for the 22.1 ha of available forested habitat (Table 1). Based on this we estimate population density of *P. oenanthe* at Pucunucho of 35 groups/km^2 and 124 individuals/km^2.

Habitat description

Using our adapted point-quarter method we sampled a total of 129 trees from 68 quadrants at 34 points, at some points trees of >20 cm CBH were not present within the cut off distance. Average radial area sampled per point was 82.3 m^2 giving a total sample area of 1.12 ha or 5.2% of the study site. The average distance between the first and second trees was 2.83 m (±2.1), average tree diameter was 18.1 cm (±11.5), average tree height was 9.7 m (±4.9) and average crown spread (Diameter) was 4.6 m (±3.53). We did not identify sampled trees except palms and *Cercropia* spp. which accounted for 5 and 4% of samples, respectively.

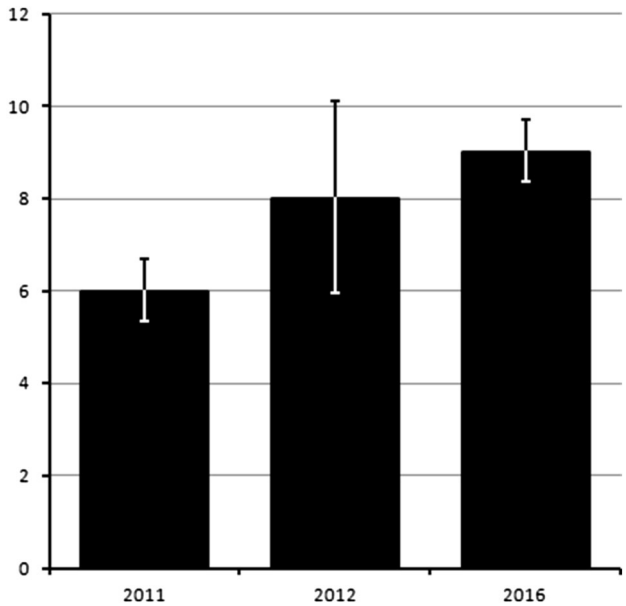

Fig. 2 Number of groups of *P. oenanthe* detected at Pucunucho during the three survey periods

Discussion

This study presents data from a rapid survey of *P. oenanthe* population density in a small fragment of secondary forest. Aside from the importance of the recovery of a population of a Critically Endangered primate species for conservation, our results provide additional base line data that, used in conjunction with density estimates from other sites and habitat types (Aldrich et al. 2008; van Kuijk et al. 2015), will aid in the proper design of conservation strategies.

The method used in this study (triangulation) provide a simple technique for rapid assessments of population densities of cryptic vocal species, particularly primates, and have been used previously on this species (Aldrich et al. 2008; van Kuijk et al. 2015) as well as on other primates (Mason 1968; Brockleman and Ali 1987; Buckley et al. 2006; Geissmann and Nijman 2006). Most population surveys undertaken in large areas of contiguous forest are extrapolated across broad areas where surveys did not take place as it is unlikely that surveys included a large fraction of potential habitat. In studies of small isolated forests it is possible to survey the entire area with minimal effort, producing robust estimates of densities and total population size without the need for extrapolation.

As basically no forest existed in the area ca. 20 years ago the population of *P. oenanthe* can be assumed to have been zero. Since its purchase for conservation in 1994 and subsequent inscription as a Private Conservation Area in the Peruvian National Protected Areas System (SINANPE), the area has undergone rapid regeneration, aided in part by enrichment planting. The first record of the return of *P. oenanthe* to the area is from 2010 although the species probably began to re-establish itself prior to this. The current estimated population size, ~27, shows a rapid repopulation of the area, by groups or individuals migrating

from outlying areas aided by natural population growth within the area.

Two studies (Aldrich et al. 2008; van Kuijk et al. 2015) report density estimates for *P. oenanthe*. Our results, from secondary forest habitat, are comparable to those of Aldrich et al. (2008) from a large forest fragment. van Kuijk et al. (2015) reported much lower densities than those reported here and by Aldrich et al. (2008). van Kuijk et al. (2015) also provide comparative density estimates for *P. oenanthe* (Table 2) between different dry forest habitat types, in their study they found higher densities for forest edge than forest interior, 49.5 and 12 ind/km^2, respectively, but higher densities in primary forest than secondary forest 19.5 and 12 ind/km^2. Differences between the habitat types of dry forest (van Kuijk et al. 2015 study) and humid forest (Aldrich et al. 2008 and this study) may affect population densities, with dry forests providing less suitable habitat for the species. Also, densities found in this study and by Aldrich et al. (2008) may be high due to natural population increase in previously un-occupied areas, coupled with a lack of dispersal opportunities. Densities could also be artificially high due to immigration of animals into these patches of restricted size, 23.5 and 74 ha respectively. In contrast, the van Kuijk et al. (2015) study was conducted in a much more extensive area of forest, 2550 ha. As several studies have suggested titi monkeys prefer natural and man-made forest edge, disturbed and secondary and regenerating forests (Peres 1993; Ferrari et al. 2000; Van Roosmalen et al. 2002) the first option seems the most likely.

Although protected, the Pucunucho area is still under threat. Two months prior to the 2012 study period, a wild fire that was probably started by neighbouring farmers to clear re-growth, destroyed some of the last remaining stands of trees in the surrounding area. This burning of lands is a common practice in northern Peru; often these fires become uncontrolled wildfires, in some cases spreading to protected areas causing substantial damage. It is possible that as a result of this fire very few *P. oenanthe* vocalizations were heard from outside the area in 2012 (one group), compared with 2011 (six groups) and 2016 (four groups), providing a source for the current high

Table 2 Comparative results from studies of titi monkeys

Species[a] and location	Method	Group size[b]	Range	Density (ind/km^2)	Habitat size	Source
P. oenanthe, Peru	Triangulation	3.75 ($N = 20$)	3–5	119	Small fragment	This study
C. oenanthe, Peru	Triangulation	3.6 ($N = 33$)	2–4	113	Small fragment	Aldrich et al. (2008)
C. oenanthe, Peru	Triangulation	2.9 ($N = 8$)	2–4	49.5 (forest edge) 12.0 (forest interior)	Extensive	van Kujik et al. (2015)
C. brunneus, Peru	Triangulation and line transect	4.6 ($N = 6$)	2–5	25–30	Extensive	Wright (2009)
C. discolour, Ecuador	Point transect with playback and home range estimate	3.5 ± 0.6	– –	47.6 ± 15.3 (point transect) 57.4 ± 12.4 (Home range estimate)	Extensive	Dacier et al. (2011)
C. cupreus, Peru	Line transect			14.6	Extensive	Bennett et al. (2001)
C. personatus, Brazil	Line transect			17	Large fragment	Müller (1996)
C. moloch, Perú	Home range	4.1	2–5	20–26	Extensive	Wright (1986)
C. torquatus, Peru	Line transect	2.9 ($N = 39$)	2–5	2.5–2.8	Extensive	Aquino et al. (2008)
C. torquatus, Colombia	Line transect direct counts	–	–	6–28 ± (Depending on habitat type)	Extensive	Defler (2013)
C. olallae, Bolivia	Fixed point	1.06 ($N = 23$)	1–5	6.3 groups	Extensive	Lopez-Strauss and Wallace (2015)
C. modestus, Bolivia	Fixed point	2.64 ($N = 21$)	1–5	13.0 groups	Extensive	Lopez-Strauss and Wallace (2015)
C. personatus, Brazil	Line transect	3.9	1–7	5.4	Extensive	Pinto et al. (1993)

[a] Genus and species as given in the study

[b] *N* number of groups

density of this species at Pucunucho through immigration of groups from the outlying area and possibly explaining some of the increase in the number of groups within the area.

A large portion of the remaining population of *P. oenanthe* is distributed across hundreds of small patches (Shanee et al. 2011; Shanee 2016). The recovery of a high density population of this species in the Pucunucho Private Conservation Area, from almost 100% deforested to secondary growth in ~20 years, provides hope for the recovery of this species if areas are set aside for mixed native species reforestation and/or natural regeneration. Enrichment planting, the aiding of natural regeneration through planting of key forest species within secondary regrowth (Parrotta et al. 1997), is another technique which can contribute to habitat recuperation (Parrotta et al. 1997) and was used successfully in Punucnucho. In this way reforestation to recuperate habitat has been suggested as a conservation strategy for other primates (Chapman and Gogarten 2012), such as the Critically Endangered kipunji (*Rungwecebus kipunji*) in Tanzania and western purple-faced langur (*Trachypithecus vetulus nestor*) in Sri Lanka (Bracebridge 2011; Rudran et al. 2013), and is possibly key to the persistence of *P. oenanthe* (Swenson et al. 2013).

As a Critically Endangered species, conservation measures are of the highest priority for *P. oenanthe*. The high population densities we found in this study, and those found in previous work on the species in fragmented areas (Aldrich et al. 2008) highlight the need to create new and maintain existing connections between populations in patches of remaining habitat. These corridors between existing populations will help ensure genetic diversity of the species and protect against anthropogenic actions and environmental stochasticity for the future. Local community hunting and deforestation bans have proven to be effective in the conservation of another Critically Endangered (*Lagothrix flavicauda*) species in northern Peru (Shanee and Shanee 2015) and should be promoted throughout the distribution of *P. oenanthe*. It is also important to undertake studies of *P. oenanthe* densities in forest fragments which contain different topographic vegetation to properly understand which physical and/or environmental factors affect this species population ecology to aid in its conservation in anthropogenic landscapes.

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