



Short Communication

New distribution records and conservation status of *Atelopus seminiferus* Cope, 1874: A Critically Endangered harlequin frog from northern Peru

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Abstract.—We provide information of the distribution, habitat, and conservation status of the harlequin frog *Atelopus seminiferus*, a poorly known species from northern Peru. Multiple individuals of *A. seminiferus* were detected inside the Alto Mayo Protected Forest, San Martín region, 87–98 km northwest from the type locality. Additionally, we used skin swab samples to test for the prevalence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), a pathogen that has been linked with population declines of harlequin frogs throughout tropical America. Our findings represent the first record of *A. seminiferus* inside a natural protected area, and we recommend an update of the IUCN Red List geographic range map of this species. Though we did not detect individuals infected by *Bd*, additional surveys are required to further assess the elevational distribution and potential for chytrid fungus infection of this Critically Endangered species.

Keywords. Bosque de Protección Alto Mayo, *Batrachochytrium dendrobatidis*, chytrid, IUCN Red List, San Martín

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Introduction

The Neotropical bufonid toad genus *Atelopus* contains 97 described species distributed across different habitats, from humid forest to paramo grassland in tropical America (Lötters 1996; Lötters et al. 2005). Of these, 69 species (71%) are categorized as Critically Endangered or Extinct under the IUCN Red List of Threatened Species (IUCN 2015). The conservation status of at least 30 species is uncertain because they remain undescribed (Coloma et al. 2010) or because a comprehensive systematic revision is required (La Marca et al. 2005; La Marca and Lötters. 2008; Lötters et al. 2011; Flechas et al. 2015). Peru contains 19 nominal species of *Atelopus* and three confirmed candidate new species from the Andes and Amazon regions (Frost 2016; Rueda-Almonacid et al. 2005). Of these, *A. loettersi*, *A. pulcher*, *A. spumarius*,

and *A. tricolor* are primarily distributed in the lowlands whereas the remaining species are restricted to elevations above 1,000 m. Montane areas along the eastern slopes of the Andes are particularly important habitat because they harbor many species of *Atelopus*. Although several species have not been seen in decades, recent field surveys have uncovered rare species such as *A. epikeisthos* (R. Santa-Cruz et al., In press). Because of this, it is essential to continue surveying these montane areas to assess if amphibian species, some of which have not been seen in many decades (e.g., Lehr and von May 2004), still exist, and to evaluate their current conservation status.

Atelopus seminiferus was described by Cope in 1874 based on a single specimen (ANSP 11383) collected by Prof. Orton from between Balsa Puerto and Moyobamba, San Martín department, northern Peru (Malnate 1971). Subsequently, this species was recorded at the Quebra-

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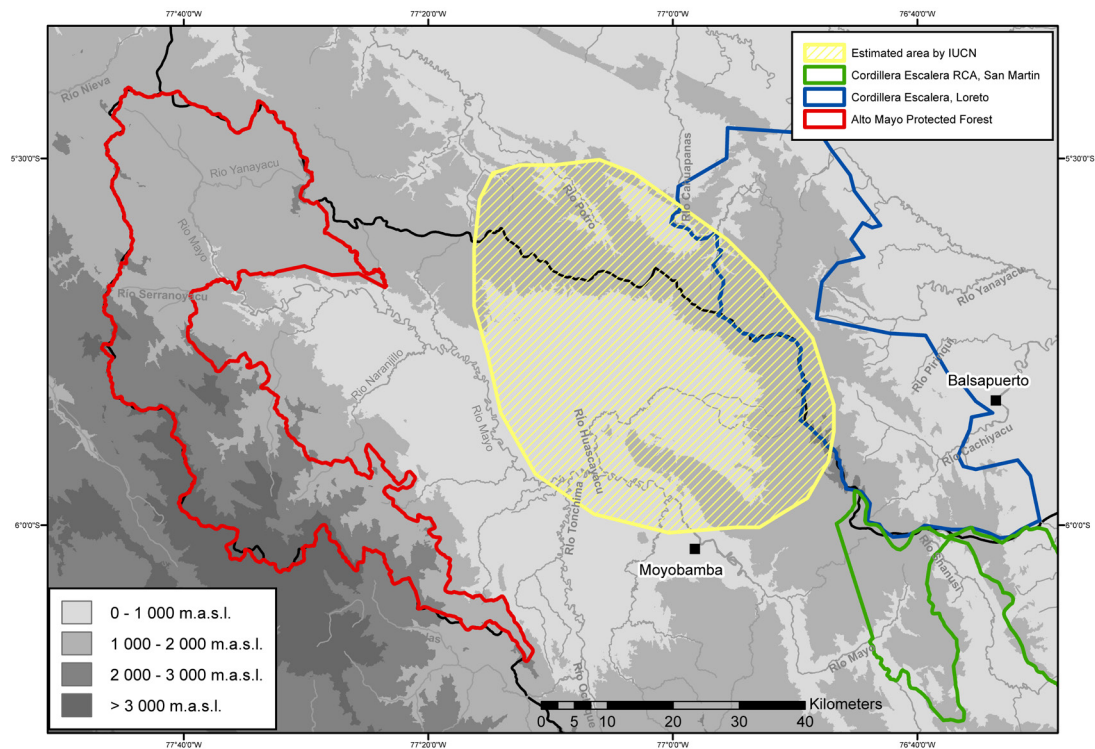


Fig. 1. Distribution of *Atelopus seminiferus* in the Mayo River basin, San Martín, Peru. Yellow polygon corresponds to geographic range estimated by IUCN. Compare with Fig. 3, which shows proposed new polygon based on results from this study. Map by Juan C. Cusi.

da Kevlada, close to an Awajun native village in Rioja Province, San Martín (Schulte et al. 2004; R. Schulte, pers. comm.). This second record was near an urban area known as Naranjos, located along the Fernando Belaunde road ($05^{\circ}44'34.05''S$, $77^{\circ}30'20.87''W$, 959 m) and ca. 7.5 km E from the boundary of Alto Mayo Protected Forest (AMPF). Subsequently, AMPF park rangers reported this species in 2007 from a site between Nuevo Eden-El Carmen villages, Rioja, San Martín (although no geographic coordinates available). In 2008, biologist Jorge Carrillo conducted field research focused on harlequin frogs (*Atelopus* spp.) at Sector Serranoyacu inside the AMPF, but no specimens were recorded in this area (J. Carrillo, pers. comm.). Recent herpetological surveys at the AMPF indicate that this reserve has at least 35 species of amphibians and 10 species of reptiles (J. Cusi et al., unpubl. data). Prior to this study, which we present here, no records of *A. seminiferus* were available from the AMPF. Thus, presenting new data on *A. seminiferus* is relevant given that recent studies of threatened amphibians from Peru did not include this Critically Endangered species (e.g., von May et al. 2008; Jarvis et al. 2015).

In this report, we provide new distributional data for *A. seminiferus* and recommend an update to the map of its known geographic distribution (Fig. 1). We also tested for the prevalence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), a pathogen that has been linked with population declines of harlequin frogs throughout tropical America (Lampo et al. 2006; Venegas et al.

2008; Flechas et al. 2015). Additionally, given that other factors such as habitat loss may have caused population declines in many other amphibian species (Catenazzi and von May 2014; Tarvin et al. 2014), we noted the type of habitat used by *A. seminiferus* in the region. Although conducting a thorough assessment of habitat change and disturbance was not a goal of the study, we provide preliminary information about habitat change and disturbance observed at some localities.

Methods and Materials

We conducted fieldwork at the Alto Mayo Protected Forest (AMPF) and Moyobamba, San Martín region, between March and December 2014 (Fig. 1). The AMPF is located along the Cordillera Oriental and is part of the upper basin of the Mayo River, northern Peru. Additionally, we surveyed around the city of Moyobamba because it is one of the type localities of *A. seminiferus*. A team of 2–3 people carried out Visual Encounter Surveys (Angulo et al. 2006; Crump and Scott 1994) during both diurnal (10:00–14:00) and nocturnal periods (18:00–00:00). Total survey effort at AMPF was 240.5 person-hours, and 16.2% of it (39 person-hours) was invested at El Carmen village, within the Venceremos sector. We collected life history data including sex, snout-vent length, and weight, as well as the type of substrate used by each individual. Additionally, we collected skin swab samples to test for prevalence of the chytrid fungus *Batrachochytrium*

Status of *Atelopus seminiferus*

Table 1. Known localities for *Atelopus seminiferus* in the basin river Mayo, San Martín, Peru. Total survey effort around El Carmen village (all localities combined) was 39 person-hours.

Locality	Map locality number	Latitude	Longitude	Elevation	Date	No. Ind.	Sex	Reference
Kevlada creek, km 393.7, near the Naranjos village	1	—	—	1100	2004	2	—	Rainer Schulte (Second record)
Trail between El Carmen and La Esperanza villages	2	5°38'48.5" S	77°41'26.0" W	1641	18/01/14	2	♂, ♀	Fredi Sangama and Florencio León*
El Arenal forest, near El Carmen village	3	5°36'26.6" S	77°41'33.1" W	1279	23/08/14	1	♀	Authors
Nueva Jordania village	4	5°34'51.2" S	77°40'50.7" W	1127	Nov. 2011	1	—	Mathieu Chouteau
El Carmen village: coffee plantations	5	5°34'57.7" S	77°41'38.2" W	1134	30/06/14	1	♀	Authors
El Carmen village	6	5°35'30.4" S	77°42'05.5" W	1243	6/10/13	1	—	Florencio León*
El Carmen village	6	5°35'30.3" S	77°42'02.1" W	1224	26/08/14	1	—	Jhonny Ramos and Elan Cachique*
El Carmen village	6	5°35'30.4" S	77°42'03.3" W	1222	30/06/14	1	♂	Authors
El Carmen Creek	6	5°35'36.2" S	77°42'06.7" W	1277	22/08/14	1	♀ gravid	Authors
El Carmen Creek	6	5°35'36.7" S	77°42'07.2" W	1267	22/08/14	1	♂	Authors
El Carmen village	6	5°35'29.1" S	77°42'03.3" W	1229	1/07/14	1	♀	Authors
Las Palmas village	7	5°37'43.6" S	77°43'57.0" W	1902	16/10/13	2	—	Florencio León*
Villa Hermosa village (Boundary Amazonas-San Martín)	8	5°32'32.4" S	77°45'49.9" W	1756	17/03/14	1	—	Marco Ramírez*

* Park rangers' names (Alto Mayo Protected Forest)

dendrobatidis (*Bd*). We took skin tissue samples using MW113-Advantage Bundling sterile cotton swabs over the abdomen, thighs, and hind limbs of each animal for a total of 30 strokes (Catenazzi et al. 2013). Skin swab samples were stored in 1.5 ml tubes. DNA was extracted from each swab and analyzed following standard protocols (Boyle et al. 2004; Hyatt et al. 2007). Additionally, we used a handheld infrared thermometer (RayTek MiniTemp MT6) to record the body temperature in vivo and the temperature of the substrate used by individuals of *A. seminiferus*. Air temperature and relative humidity were measured every 15 minutes during 24 hours with one HOBO U23 Pro v2 data logger (Onset) at one of the survey sites. Given that, at the time of the study, only the holotype was available in a museum collection (Academy of Natural Sciences, Philadelphia [ANSP] in North America), four specimens were collected (MUSM 33328, 33327, 33662, JCM H-24) and deposited as reference material at the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM) in Lima, Peru. For this purpose, a research and collecting permit (RJ N° 001-2014-SERNANP-BPAM-JEF) was obtained from Peru's Ministerio del Ambiente.

Results

We found individuals of *A. seminiferus* at six localities within of the Alto Mayo Protected Forest: 1) trail between El Carmen and La Esperanza villages, 2) El Arenal forest near El Carmen village, 3) Nueva Jordania village, 4) El Carmen village, including the actual village, a creek, and coffee plantations, 5) Las Palmas village, and 6) Villa Hermosa village (Table 1). All of these localities fall outside the IUCN range map polygon (<http://>

maps.iucnredlist.org/map.html?id=54548, accessed on 8 August 2016; Fig. 1). Most individuals observed in the field were detected around El Carmen, a small village inhabited primarily by coffee farmers and surrounded by coffee plantations. We also obtained photographic records from Las Palmas and Villa Hermosa, provided by park rangers who found *A. seminiferus* during patrols and surveillance against illegal logging of the forests. Even though we did not visit Nueva Jordania, Mathieu Chouteau (pers. comm.) informed us about the record of one specimen in this locality (Table 1). The complete set of these new localities was used to calculate the extent of occurrence. Using ArcGIS, we estimated that the Extent of Occurrence of *A. seminiferus* is ca. 2,520 km².

We recorded 14 adult individuals at the AMPF between March and December on 2014. Most adult individuals were found on leaf litter between 10:00 h to 14:00 h (MUSM 33328, 33327, 33662), and some individuals were found at night along the margins of a creek; these individuals were sitting on top of fern leaves near the ground. Although we did not hear vocalizations of *A. seminiferus*, one mating pair was photographed on 18 January 2014 (Fig. 2A) and one gravid female (field number JCM H-24) was found on 22 August 2014. Advertisement calls and tadpoles of this species remain unknown. Coloration pattern coincides with the description of Lötters and Schulte (2005): dorsal surface uniformly velvety black with minute yellow or pinkish cream dots scattered throughout the dorsum, forelimbs and hindlimbs; some individuals possess pinkish dots on lower jaw; palmar and plantar surfaces dark red; belly pink in males and dark red in females, over a black background in both sexes; throat with pink blotches over a black background; iris black with yellow ring around pupil (Fig. 2 B–D).

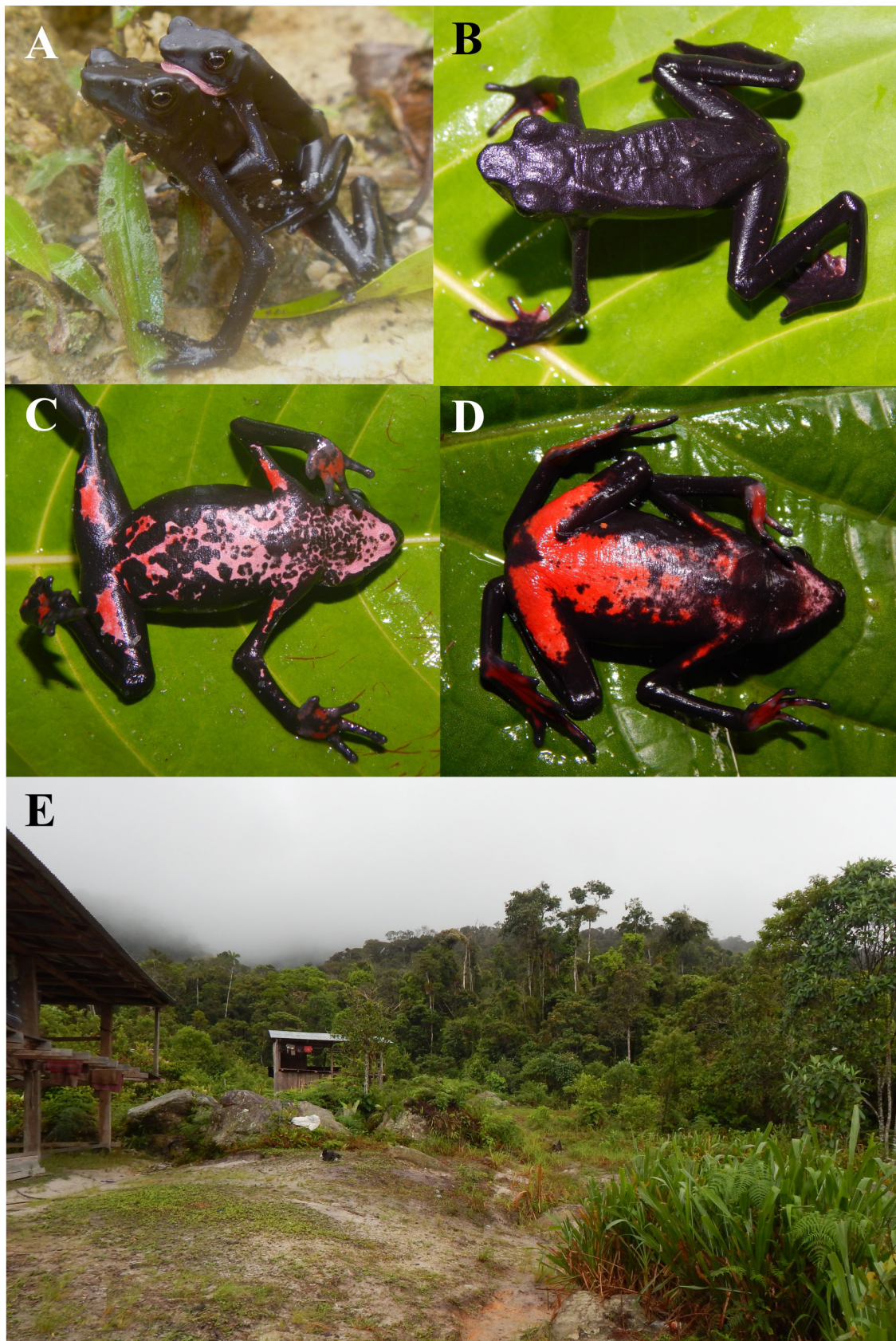


Fig. 2. (A) A pair of *Atelopus seminiferus* in amplexus, found between El Carmen and La Esperanza [not collected]. Photo by Fredi Sangama and Florencio León. (B) Dorsal coloration pattern of a female MUSM 33328. (C) Ventral coloration pattern in a male MUSM 33327. (D) Ventral coloration pattern in a female MUSM JCM H-24. (E) El Carmen village in Alto Mayo Protected Forest, Rioja province, San Martín. (E). Photos B–E by Juan C. Cusi.

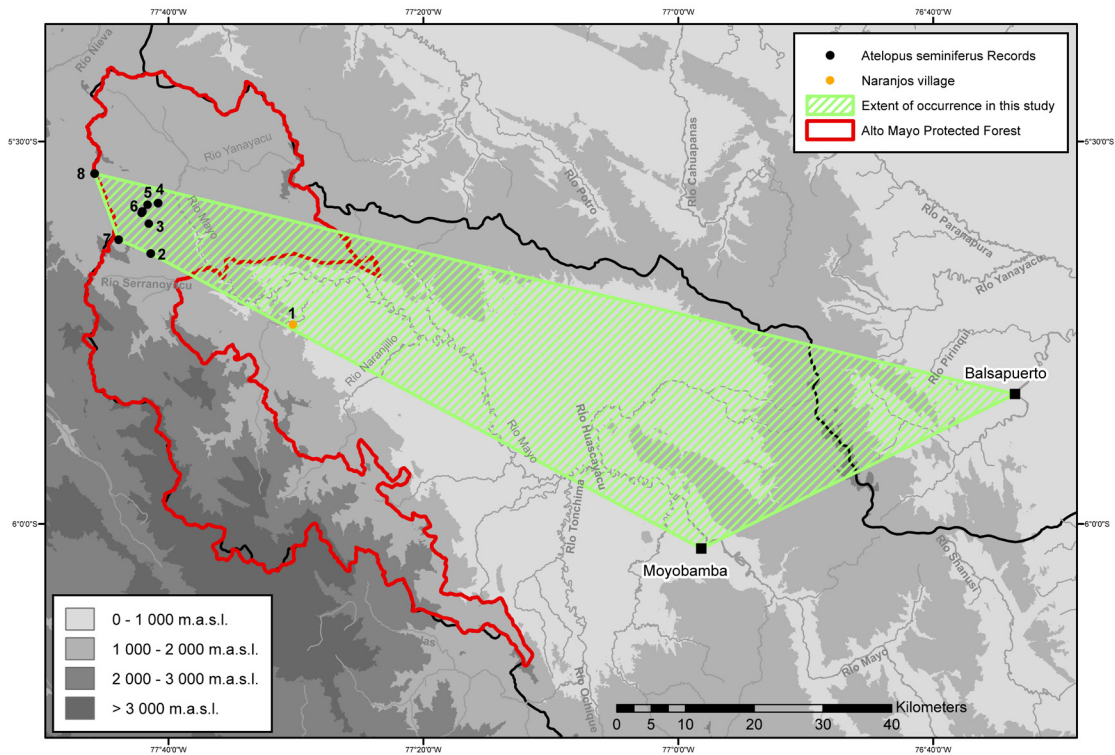


Fig. 3. Updated distribution map of *Atelopus seminiferus*. Black dots indicate new localities reported in this study. Light green area corresponds the estimated Extent of Occurrence (ca. 2,520 km²) based on the new records presented here and the previously known localities. Numbers correspond to labels in Table 1. Map by Juan C. Cusi.

Our *Bd* prevalence assays were negative for the presence of the chytrid fungus (*Bd*) in samples from El Carmen (*Bd* negative, $n = 5$). Mean body temperature was 19.9 °C, and temperatures of air and substrate were 21.2 °C and 21.3 °C ($n = 4$), respectively. We recorded climatic parameters in one primary forest near El Carmen (locality number 6 in Fig. 1 and Table 1) on 27–28 June 2014. Mean air temperature during the day was 18.48 ± 2.14 °C and relative humidity was $96.89 \pm 2.67\%$. Mean air temperature at night was 15.90 ± 1.19 °C and relative humidity was $98.16 \pm 2.41\%$. Using the new records and the previously know localities, we created a polygon to update the geographic distribution map of *A. seminiferus* (Fig. 3).

Discussion

Our study documents the existence of populations of *A. seminiferus* inside the Alto Mayo Protected Forest, and it represents the first record of this Critically Endangered species inside a natural protected area. The new localities reported here represent an extension of the geographic range of *A. seminiferus* by ca. 45 km west from the western boundary of the geographic range recognized by IUCN (Fig. 1). Specifically, the new localities are ca. 23.1 km northwest from Naranjos and 86.7 km northwest from Moyobamba (type locality). The record from Villa Hermosa (Table 1) represents the northernmost locality known to date for this species. Therefore, we recommend

an update of the IUCN Red List geographic range map of this species. Concretely, we recommend that the new polygon generated here (Fig. 3) should be considered in the next IUCN Red List assessment and replace the currently available polygon. As with most harlequin frogs, *A. seminiferus* is considered a rare species given that very few specimens have been observed and collected in the wild. The IUCN Red List assessment (Schulte et al. 2004) states that data on population status or abundance were not available and emphasized that additional field surveys were needed in the region. In addition to detecting *A. seminiferus* at six new localities (i.e., sites located >1 km apart from each other), our findings suggest that this species has a fragmented distribution. Using the IUCN Red List criteria (IUCN 2016), which indicates that if a species is known from fewer than ten threat-defined locations and the extent of occurrence is smaller than 20,000 km², it should be classified as Vulnerable or Endangered. *Atelopus seminiferus* is known from eight localities (Table 1), has an estimated Extent of Occurrence (EOO) of 2,520 km²; additionally, the estimated EOO and the number of known subpopulations or locations has varied over time (with a total of 16 individuals detected in 10 years). Therefore, we suggest that *A. seminiferus* might be classified as Vulnerable B1ac(i,iii).

Our field surveys indicate that *A. seminiferus* inhabits primary montane forests and might tolerate some level of disturbance given that some individuals were found in modified forested habitats. In particular, *A. seminiferus*



Fig. 3. Dorsal and ventral views of the holotype of *Atelopus seminiferus* (ANSP 11383), deposited in the herpetological collection at the Academy of Natural Sciences of Drexel University, Philadelphia. Photos courtesy of Ned Gilmore.

occurs in areas surrounding El Carmen (Fig. 2E), where native montane forests have been cleared and replaced by subsistence agricultural plantations (coffee, pineapple, and banana) and areas used by livestock (cattle, horses, and mules). However, it would be premature to assume that populations of *A. seminiferus* in disturbed areas will persist on the long term, given that pesticides and fertilizers used in agricultural plantations may have negative effects on amphibians (Hayes et al. 2002). Furthermore, habitats used by *A. seminiferus* appear to be impacted by the expansion of human settlements associated with urban development in Moyobamba and Balsapuerto in recent years. Given the geographic proximity to Moyobamba-Balsapuerto, it is possible that *A. seminiferus* also occurs at Cordillera La Escalera Regional Conservation Area (RCA), in San Martín region, and Cordillera Escalera, in Loreto region; Fig. 1. However, the species has not been detected in this protected area (Pitman et al. 2014). The chytrid fungus (*Bd*), a pathogen associated with massive declines of amphibians around the world (Catenazzi et al. 2011; Lips et al. 2008; Vredenburg et al. 2010), has been assumed to be a possible threat for *A. seminiferus*. Although we did not detect *Bd*-infected individuals, further monitoring of populations of *A. seminiferus* and larger skin swab sample sizes are needed to test if the pathogen is affecting any of these populations more widely. Continuous assessment of *Bd* prevalence is essential given that chytridiomycosis has likely affected many species of *Atelopus* (Bonaccorso et al. 2003; La Marca et al. 2005; Lampo et al. 2006; Lips et al. 2008). The new voucher specimens collected during this study will be useful for future morphological studies, especial-

ly because the only available type material (the holotype, ANSP 11383) has deteriorated and has broken phalanges on left hand and right foot (Fig. 4). In summary, our findings provide valuable insights on the conservation status of *A. seminiferus* and an updated map of the known geographic range of this species.

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