



INSTITUTO POLITÉCNICO NACIONAL

**Centro Interdisciplinario de Investigación para el Desarrollo
Integral Regional, Unidad Oaxaca**

**Maestría en Ciencias en Conservación y
Aprovechamiento de Recursos Naturales
(Biodiversidad Del Neotrópico)**

**Patrones de movimiento de *Incilius spiculatus*
(Anura: Bufonidae) en bosque mesófilo de montaña
con distinto grado de perturbación**

TE S I S

QUE PARA OBTENER EL GRADO DE:

MAESTRO EN CIENCIAS

PRESENTA:

BIOL. MEDARDO ARREORTÚA MARTÍNEZ

DIRECTORA:

DRA. EDNA LETICIA GONZÁLEZ BERNAL

Santa Cruz Xoxocotlán, Oaxaca, México agosto 2020



INSTITUTO POLITÉCNICO NACIONAL

SECRETARIA DE INVESTIGACIÓN Y POSGRADO

ACTA DE REGISTRO DE TEMA DE TESIS Y DESIGNACIÓN DE DIRECTOR DE TESIS

Ciudad de México, 18 de mayo del 2020

El Colegio de Profesores de Posgrado de **CIIDIR UNIDAD OAXACA** en su Sesión
(Unidad Académica)

Ordinaria No. 5 celebrada el día 18 del mes mayo de 2020 conoció la solicitud presentada por el (la) alumno (a):

| | | | | | |
|-------------------|-----------|-------------------|----------|-------------|---------|
| Apellido Paterno: | Arreortúa | Apellido Materno: | Martínez | Nombre (s): | Medardo |
|-------------------|-----------|-------------------|----------|-------------|---------|

Número de registro: A 1 8 0 1 7 1

del Programa Académico de Posgrado: Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales

Referente al registro de su tema de tesis; acordando lo siguiente:

1.- Se designa al aspirante el tema de tesis titulado:

“Patrones de movimiento de *Incilius spiculatus* (Anura: Bufonidae) en bosque mesófilo de montaña con distinto grado de perturbación”

Objetivo general del trabajo de tesis:

Estudiar el movimiento de una especie de anuro endémica de ciclo de vida bifásico a través de dos niveles de perturbación dentro de su hábitat natural de bosque mesófilo y evaluar los posibles impactos generados por las alteraciones humanas sobre el desplazamiento de esta especie.

2.- Se designa como Directores de Tesis a los profesores:

Director: Dra. Edna Leticia González Bernal 2° Director:
No aplica:

3.- El Trabajo de investigación base para el desarrollo de la tesis será elaborado por el alumno en:

La fase de laboratorio se llevó a cabo en el laboratorio de ecología de anfibios dentro del Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca; La fase de campo se realizó en la comunidad de Santa Cruz Tepetotutla en el municipio de San Felipe Usila, Oaxaca

que cuenta con los recursos e infraestructura necesarios.

4.- El interesado deberá asistir a los seminarios desarrollados en el área de adscripción del trabajo desde la fecha en que se suscribe la presente, hasta la aprobación de la versión completa de la tesis por parte de la Comisión Revisora correspondiente.

Director(a) de Tesis

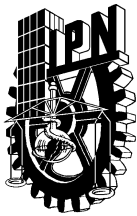
2° Director de Tesis (en su caso)

Dra. Edna Leticia González Bernal
Aspirante

Presidente del Colegio

Arreortúa Martínez Medardo

Dr. Salvador Isidro Belmonte Jiménez



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SIP-14

REP 2017

ACTA DE REVISIÓN DE TESIS

En la Ciudad de Santa Cruz Xoxocotlán, Oaxaca siendo las 14 horas del día 22 del mes de julio del 2020 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Posgrado de: Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca. Para examinar la tesis titulada: "Patrones de movimiento de *Incilius spiculatus* (Anura: Bufonidae) en bosque mesófilo de montaña con distinto grado de perturbación"

del alumno:

| | | | | | |
|--------------------------|-----------|--------------------------|----------|--------------------|---------|
| Apellido Paterno: | Arreortúa | Apellido Materno: | Martínez | Nombre (s): | Medardo |
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Aspirante del Programa Académico de Posgrado: Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales.

Una vez que se realizó un análisis de similitud de texto, utilizando el software anti plagio, se encontró que el trabajo de tesis tiene 3% de similitud. **Se adjunta reporte de software utilizado.**

Después que esta Comisión revisó exhaustivamente el contenido, estructura, intención y ubicación de los textos de la tesis identificados como coincidentes con otros documentos, concluyó que en el presente trabajo **SI** **NO** **SE CONSTITUYE UN POSIBLE PLAGIO.**

JUSTIFICACIÓN DE LA CONCLUSIÓN:

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Satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

COMISIÓN REVISORA DE TESIS

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INSTITUTO POLITÉCNICO NACIONAL
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CARTA CESION DE DERECHOS

En la Ciudad de Oaxaca el día 30 del mes julio del año 2020, el que suscribe **Medardo Arreortúa Martínez** alumno del Programa de Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales, con número de registro **A180171**, adscrito al Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca, manifiesta que es autor intelectual del presente trabajo de Tesis bajo la dirección de la Dra. Edna Leticia González Bernal y cede los derechos del trabajo titulado: Patrones de movimiento de *Incilius spiculatus* (Anura: Bufonidae) en bosque mesófilo de montaña con distinto grado de perturbación, al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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Medardo Arreortúa Martínez
Nombre y firma

AGRADECIMIENTOS

A la comunidad de Santa Cruz Tepetotutla por la hospitalidad y el apoyo en el desarrollo de este trabajo y en reconocimiento por su esfuerzo en la conservación de la biodiversidad de su territorio.

A mi directora: Dra. Edna González Bernal

A los miembros del comité revisor de este trabajo

A mis amigos y compañeros del laboratorio de Ecología para la Conservación de Anfibios por el apoyo en el desarrollo de esta investigación.

A mi familia que siempre me ha apoyado. A mi madre a quien ha sido parte importante de este logro. A mi novia Mayra por su apoyo y paciencia

Y en memoria de un gran amigo y compañero

Eugui Roy Martínez Pérez

INTRODUCCIÓN GENERAL

Los anfibios son el grupo de vertebrados mayormente amenazados y en declive a nivel mundial. (Collen *et al.*, 2009; Stuart *et al.*, 2010). Las principales causas de su declinación son los cambios ambientales, las enfermedades emergentes, la introducción de especies invasoras y principalmente la fragmentación y destrucción del hábitat (Young *et al.*, 2004; Ficetola, 2015).

La pérdida de hábitat en particular ha generado impactos negativos en la vida silvestre, en particular en el movimiento animal, el cual es un factor importante para su supervivencia y conservación (Wells, 2007 & Pittman *et al.*, 2014).

A pesar de esto, el impacto que la pérdida de hábitat tiene sobre los anfibios en relación a su movimiento ha sido poco evaluado ya que se supone, las habilidades de dispersión de este grupo son bajas debido a sus características biológicas como; su tamaño y su piel altamente permeable (Cushman, 2006). Este concepto erróneo ha causado que se pierda la atención sobre un proceso crucial para comprender la declinación de un grupo altamente vulnerable como lo es el movimiento. Entender este aspecto ecológico es necesario para la conservación de este grupo, particularmente en aquellas especies con ciclos de vida bifásicos en constante interacción y movilidad entre ambientes acuáticos y terrestres (Wells, 2007).

El presente trabajo de tesis ha sido dividido en dos capítulos. El primer capítulo está enfocado al tema central de la investigación el cual se basa en evaluar los patrones de movimiento de *I. spiculatus* en relación a la perturbación del hábitat producida en un bosque mesófilo de montaña en Oaxaca.

El segundo capítulo trata sobre aspectos de la historia natural de esta especie recabados de forma paralela durante el trabajo de campo de este mismo proyecto. Además, incluye hábitos reproductivos y la descripción del renacuajo de *Incilius spiculatus* descubierto en este periodo. Este capítulo ya ha sido enviado para su publicación.

El fin de esta investigación es contribuir al entendimiento de la ecología de movimiento de los anfibios en general así como a la conservación de una especie nativa de anfibio en peligro. Esto mediante el aporte al entendimiento de su vagilidad, los posibles efectos de la perturbación sobre la misma y mediante el conocimiento de su historia natural, lo cual es determinante para diseñar planes de conservación.

CAPITULO I

Patrones de movimiento de *Incilius spiculatus* (Anura: Bufonidae) en bosque mesófilo de montaña con diferentes grados de perturbación.

Movement patterns of *Incilius spiculatus* (Anura: Bufonidae) in montane cloud forest with different disturbance degrees.

1 **Movement patterns of *Incilius spiculatus* (Anura: Bufonidae) in montane cloud**
2 **forest with different disturbance degrees**

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10 **Abstract:**

11 Animal movement is an important component of individual survival, fitness, gene flow and
12 population maintenance. For vulnerable groups such as amphibians, particularly those with a
13 biphasic life cycle, understanding their vagility results imperative when interpreting their
14 interaction with their habitat, specially of those that are distributed within ecosystems with high
15 human pressure. We aimed to study the movement patterns of a terrestrial amphibian (*Incilius*
16 *spiculatus*) within montane cloud forest with different perturbation degrees. Between March and
17 October 2018 and February to September 2019, we monitored 30 individuals of this species by
18 radiotelemetry. We obtained distance moved per individual by site type and seasons. Home range
19 was estimated by the minimum convex polygon (MCP) and fixed kernel (KD) methods. However,
20 these estimates do not show differences between sites or sexes. On other hand, a shift in distances
21 moved by site and sex were found. Perturbation causes an increase in distances moved, while in
22 general females move longer distances in comparison to males. Also, perturbation had an effect on
23 shelter use between sites. With this information we aim to add to the understanding of the
24 interaction between habitat disturbance and amphibian movement.

25 **Key words:** Vagility, home range, fragmentation, disturbance, ecology of movement, habitat use.

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31 **Resumen**

32 El movimiento animal es un componente importante para el estado físico, el flujo de genes la
33 supervivencia individual y el mantenimiento de las poblaciones. En grupos vulnerables en especial
34 aquellos con un ciclo de vida bifásico, como los anfibios, comprender su vagilidad es fundamental
35 para entender las interacciones con su hábitat, en especial en aquellos que se distribuyen en
36 ecosistemas con alta presión humana. Nuestro objetivo fue evaluar los patrones de movimiento de
37 un anfibio (*Incilius spiculatus*) dentro de un bosque mesófilo de montaña con diferentes grados de
38 perturbación. Entre marzo y octubre del 2018 y febrero y septiembre de 2019 monitoreamos 30
39 individuos por medio de radio telemetría con lo cual se obtuvieron las distancias recorridas
40 individuales y por estaciones. Estimamos el tamaño del ámbito hogareño utilizando el método del
41 polígono mínimo convexo (MCP) y el método de densidad de kernel fijo (KD) sin embargo, no
42 encontramos diferencias entre el tamaño por sitios o sexos. Logramos encontrar que existen
43 diferencias entre las distancias recorridas por sexo donde las hembras tienden a moverse trayectos
44 más largos que los machos. Además, observamos que la perturbación tiene un efecto sobre el uso
45 de los refugios entre sitios y provoca que las distancias recorridas aumenten. Con esta información,
46 nuestro objetivo es incrementar la comprensión de la interacción entre la alteración del hábitat y el
47 movimiento de anfibios.

48 **Palabras clave:** Vagilidad, ámbito hogareño, fragmentación, perturbación, ecología de
49 movimiento, uso de hábitat.

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INTRODUCTION

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59 Movement is one of the main processes involved in animal survival (Wells, 2007 and Pittman et
60 al., 2014). Animals need to move to find food, water, refuge, reproduction sites and partners, among
61 other resources (Henrique, 2017; Powell, 1999; Semlitsch, 2008; Spiegel et al., 2017; Tucker et al.,
62 2018). In addition this usually involves decisions that can either put an individual at risk (i.e. by
63 increasing their mortality by exposure to predators) or compromise its energy costs (by making
64 them invest more reserves in moving) (Carr & Fahrig, 2001; Amo et al., 2006, 2007).

65 Even when movement importance in animal survival has been investigated, its understanding under
66 several scenarios and vertebrate groups, still remains poor (Nathan et al., 2008; Shepard et al.,
67 2013). One of the main aspects that influence animal movement is landscape composition, being
68 those ecosystems under human pressure due to habitat transformation, the ones that represent a
69 higher challenge for several species ((Rothermel & Semlitsch, 2002). Habitat transformation keeps
70 occurring rapidly around the world and it is considered the first factor causing animal declines
71 worldwide (Foley et al., 2005). These declines are usually related to the way altered landscape
72 conditions interfere with animal movement or affect their home ranges' size (Tucker et al., 2018).

73 The effects of habitat loss on animals can vary with size, sex, age, and biology. Thus its effects on
74 different groups might not always be extended to other species. For example, it has been recorded
75 in small mammals (*Pteromys volans*) that, within a matrix of urbanized landscape, home range
76 vary between sexes, because the females seek specific characteristics for breeding sites and due to
77 territoriality, they remain closer to their nests (Mäkeläinen et al., 2015). In addition, smaller
78 individuals usually have smaller home ranges that can still be functional at levels of habitat loss
79 that for bigger animals results impossible, on the other hand, the reduction of their home ranges
80 can generate competition for space between species with the same size. For example when habitat
81 reduction triggers competition for nest availability between two species (*Cercartetus nanus* and
82 *Antechinus stuartii*) of small mammals (Blandon et al., 2002; Tucker et al., 2018).

83 For amphibians, habitat transformation through the reduction of food, refuge availability and by
84 altering environmental conditions, forces organisms to change movement patterns or to modify
85 migration routes in some cases even within open areas which increases their predation risk
86 (Cushman, 2006; Semlitsch, 2008). Another common consequence of habitat loss is the
87 interruption of connectivity between terrestrial and aquatic habitats, the first of relevance as
88 providers of refuges during the dry season, the second as providers of reproductive sites generally
89 during the rainy season (Ficetola, 2015; Young et al., 2005). In addition, due to their biological
90 characteristics, amphibians present particular limitations in terms of displacement, one of the main
91 ones being their constant need for water, since their skin is highly permeable they are susceptible
92 to desiccation, which often limits their migrations to periods of high humidity such as the rainy

93 season (Russell et al., 2005a; Sinsch, 1990). Despite, amphibian movement has been understudied
94 since it has been assumed that this group presents low dispersal abilities. This misconception has
95 caused a gap of knowledge about a process that is crucial when aiming to understand amphibian
96 population declines: movement in relation to habitat transformation. This gains relevance for those
97 species that due to a biphasic life cycle have to move between terrestrial and aquatic ecosystems.

98 We studied movement patterns of a terrestrial amphibian with a biphasic life cycle, *Incilius*
99 *spiculatus*. Since it is an amphibian that inhabits primary cloud forest, we expect to find changes
100 in moved distances, habitat use and home range when comparing habitats with different degrees of
101 disturbance: a primary mountain cloud forest and secondary vegetation with constant human
102 intervention. Understanding the ways in which this species copes with habitat transformation could
103 lead to identify the challenges posed by habitat modification when moving to/from reproductive
104 sites or simply when transformation occurs in its range, an important aspect under the amphibian
105 crisis scenario. This will allow us to know the possible impacts generated by human alterations in
106 the displacement of this species and will help us understand how the disturbance alters terrestrial
107 amphibian movement patterns.

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METHODS

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113 **Study species**

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115 *Incilius spiculatus* is an endemic toad of the Sierra Madre in Oaxaca categorized as endangered
116 (EN) according to the red list of threatened species (IUCN, 2016). It presents a biphasic life cycle,
117 with a stream-breeding behaviour during the dry season as other species of the genus. (Arreortúa
118 et al., in process). Its altitudinal distribution ranges from 600 to 1,700 masl., being primary
119 vegetation of mountain cloud forests its natural habitat (Mata-Silva et al., 2015; Mendelson, 1997).
120 The habitat use and spatial relationships of this species are still unknown.

121

122 **Study area**

123

124 Santa Cruz Tepetotutla is located in the Sierra Madre of Oaxaca in Oaxaca México, (17.738709°N
125 y -96.558424°O) (FIG 1) at an altitude of 1150 masl. It presents an average temperature of 15 °C
126 to 36 °C and is characterized by having two seasons, the dry season that goes from January to May
127 and the wet season from June to October. The average precipitation is 13 mm and 260 mm
128 respectively (CONABIO 2017). The vegetation is composed of montane cloud forest, evergreen
129 forest and oak forest (INEGI 2017).

130 The community of Santa Cruz has a protects, under the Indigenous and Community Conserved
131 Areas (ICCA's) modality, around 9,570 Ha of primary and secondary montane cloud forest. This
132 allows to find patches of forest with different disturbance degrees, a crucial aspect for the
133 development of this project. With the aim of establishing different conditions that *I. spiculatus*
134 might face due to habitat disturbance and determine how this affects its mobility and habitat use,
135 we selected two areas with different perturbation degrees (Table 1). From now on, only for practical
136 purposes, we will use the term "conserved site" to refer to primary montane cloud forest, and
137 "disturbed site" to refer to secondary montane cloud forest.

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142 **Radio tracking**

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144 Toad movement was monitored using radio telemetry VHF (very high frequency) and transmitters
145 (Telenax model TXC-004T) that weigh less than 10% (0.7g - 1g) of the focus organism's weight.

146 Toads were searched by the visual encounter's technique (Heyer 1994) within the selected sites.
147 Once a toad was located, we recorded its sex, age, body length (SVL), hindlimbs length, weight,
148 time and activity at the capture moment and location. Radio transmitters were attached to the pelvic
149 girdle with a nylon and polyethylene harness to avoid causing any harm (Rathbun & Murphey,
150 1996). Afterwards toads were released at the capture site and radio tracking started an hour later to
151 reduce effects of possible stress due to manipulation on their behavior.

152 Monitoring of *I. spiculatus* was carried out during the dry season (from February to May); and the
153 wet season (from July to October) during 2018 and 2019 and among two forest types: primary
154 montane cloud forest and secondary montane cloud forest. Toads were followed from 3 to 7 days
155 per site, based on transmitter batteries' duration. Searches were made in lapses of 2 hours from 9:00
156 am to 2:00 am. Each recapture location was recorded (Garmin GPSMAP-64st) in addition to body
157 temperature, encounter time, activity, microclimatic data (humidity, ambient temperature) and data
158 of surrounding vegetation.

159 To avoid pseudo replication we marked all individuals with visible implant elastomer tags which
160 are a minimally invasive and an effective method already tested in amphibians (Measey et al.,
161 2001; Osbourn et al., 2011).

162

163 **Movement parameters**

164

165 Distances covered point by point at each encounter were calculated using the "measure" tool in
166 ArcGis 10.4 (ESRI). Individual distances were obtained by the sum of all successive movements
167 throughout the monitoring. Movements were categorized as: local adjustments, those that ranged
168 from 0m to 5m and exploratory movements, those longer than 5m. This categorization was carried
169 out with the aim of defining the type of movement as a function of distance, that is, local
170 adjustments reflect greater activity within a small area, unlike exploratory movements that are
171 identified as searching migrations of new resources (Marshall et al., 2006; Benhamou & Cornélis,
172 2010).

173 Movement data were standardized as the distance traveled in 24-hour cycles. The number of
174 encounters within a shelter were considered as 0m and were used to estimate movement proportions

175 per site. Differences in movement between sexes and seasons were analyzed with non-parametric
176 statistical tests, Kruskal-Wallis (ANOVA).

177

178 **Morphology and movements**

179

180 Body condition index (BCI) was calculated through the residuals of a simple linear regression
181 between SVL and body weight (Jakob et al., 1996; Rodríguez-Prieto et al., 2011).

182 Subsequently, an analysis of variance (ANOVA) was performed to compare the BCI between the
183 sites to determine a possible effect of disturbance degree. To evaluate if BCI is related to distances
184 traveled, we made a linear regression.

185 We used a simple linear regression with data on the total distances traveled for each individual and
186 the measurements of the hind legs, to evaluate the distance in relation to the size of the limbs
187 (Phillips et al., 2006).

188

189 **Habitat use**

190

191 Every time a toad was located, the following data was recorded: surrounding vegetation (herbaceous,
192 shrubs and trees coverage percentage), canopy cover and substrate coverage percentage (rocks, leaf
193 litter, soil). In parallel, we registered the same data at unused sites.

194 Refuge use was identified and recorded every time a toad was found sheltered. Shelter type was
195 categorized as: holes formed by external tree roots, burrows, leaf litter and fern patches. The
196 number of encounters within a shelter were recorded and compared among sites by a chi squared
197 test.

198

199 **Home range estimation**

200

201 To try to avoid overestimation of the kernel method, we try to implement the method presented by
202 Benhamou and Cornelis (2010), with which limits and unused areas can be identified by the type
203 of movement. Although the authors recommend not using this method without strict data on the

204 activity of the organisms, our monitoring based on direct observations gives us the certainty of the
205 periods of refuge and active movement, so it was decided to add weight to the field. of the distance
206 traveled (Benhamou and Cornélis, 2010).

207 Home range (HR) was calculated per individual using the extension Hawth's Tools and Minimum
208 Convex Polygon method (MCP) (ArcGis 9.3 ESRI). Only individuals with more than three
209 relocations were considered.

210 We also used the fixed kernel method with 50% contours to define the core areas of activity and
211 95% to estimate the total scope of the home range environment (Seaman et al., 1998 & Powell,
212 2000). Taking in consideration species' characteristics, the model smoothing factor was adjusted
213 to 10 m.

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RESULTS

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220 **Movement parameters**

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222 We tracked a total of 30 individuals: six females, ten males and two juveniles at conserved sites
223 and eight females, 3 males and 1 juvenile at disturbed sites.

224 Distances moved differed between sexes (FIG 2). Female toads moved longer distances than males
225 (Females $\bar{x} = 36.40 \pm 3.40$; Males $\bar{x} = 20.46 \pm 3.67$. Kruskal Wallis $X^2_{(1,24)} = 6.6138$, $P = 0.0101$),
226 independently of the place (conserved or disturbed).

227 When evaluating distances moved at both site types, we found that toads at perturbed sites moved
228 longer distances (Conserved $\bar{x} = 5.146 \pm 0.497$ Disturbed $\bar{x} = 7.150 \pm 0.616$; Kruskal-Wallis $X^2_{(1,444)}$
229 $= 7.425$, $P = 0.006$) and also presented longer local adjustments at those same sites (Conserved $\bar{x} =$
230 0.49 ± 0.109 ; Disturbed $\bar{x} = 1.035 \pm 0.146$. Kruskal Wallis $X^2_{(1,258)} = 11.032$, $P = 0.009$) (FIG 3).
231 Seasonally, it's during the rainy season when toads moved longer distances, a pattern close to
232 significance (Rain $\bar{x} = 6.199 \pm 0.411$; Dry $\bar{x} = 3.76 \pm 1.180$; Kruskal-Wallis $X^2_{(1,444)} = 3.367$, $P = 0.05$)
233 (FIG 4).

234 When analyzing the proportion of organisms sheltered at each site, we found that disturbed sites
235 present more activity, in other words individuals spend less time in a refuge at disturbed sites ($X^2_{(1)}$
236 $= 14.633$, $P = 0.0001$). In addition, the females are the ones that tend to be more active
237 independently of the site ($X^2_{(1)} = 13.88$; $P = 0.0003$).

238

239 **Morphology and movement**

240

241 Tibia length had an effect on distances moved by toads, being the individuals with longer legs the
242 ones that moved longer distances ($r^2 = 0.9985$ $P > 0.0348$). In addition, individuals at perturbed
243 sites had longer legs ($F_{(1,25)} = 6.5766$ $P = 0.01$) and longer SVL ($F_{(1,25)} = 8.8273$ $P = 0.02$) without
244 this being related to sex $P > 0.05$.

245 There were no differences in body condition neither between individuals from each site ($P > 0.6$)
246 nor among sexes ($P > 0.5$). Body condition did not have an effect on distances moved either
247 ($R = 0.0703$ $P = 0.1905$).

248 **Habitat use**

249
250 We found that disturbance has an effect on shelter selection by toads ($X^2_{(3)}=65.801$; $P<0.0001$).
251 Toads at conserved sites used more frequently leaf litter (54%) and holes formed by external tree
252 roots (34.50%) and in less proportion burrows and fern patches while at perturbed sites, ferns
253 patches (38.1%) were the preferred refuge followed by leaf litter (33.3%) (FIG 5).

254 Environmental temperature changed with perturbation being perturbed sites warmer (Conserved
255 $\bar{x}=18.33 \pm 0.1540$; Disturbed $\bar{x}=20.86 \pm 0.2208$; Kruskal Wallis $X^2_{(1,366)}=68.268$. $P<0.0001$) and
256 with lower relative humidity (Conserved $\bar{x}=91.139 \pm 0.56$; Disturbed $\bar{x}=73.83 \pm 0.86$; Kruskal
257 Wallis $X^2_{(1,367)}=153.61$. $P<0.0001$)

258

259 **Home range estimation**

260

261 HR size (table 1) did not change with perturbation, neither when calculated by the MCP (Conserved
262 $\bar{x}=363.80 \pm 141.43$; Disturbed $\bar{x}=748.923 \pm 173.21$. Kruskal-Wallis $X^2_{(1,23)}=1.6727$, $P=0.2020$)
263 nor by kernel analysis' core area KD 50% (Conserved $\bar{x}=360 \pm 65.16$; Disturbed $\bar{x}=367 \pm 78.31$.
264 Kruskal-Wallis $X^2_{(1,20)}=0.0011$, $P=0.9734$) and KD 95% (Conserved $\bar{x}=1229.69 \pm 130.06$;
265 Disturbed $\bar{x}=1416.15 \pm 150.18$. Kruskal-Wallis $X^2_{(1,26)}=0.6983$, $P=0.4034$). However, HR areas
266 (Ha) are slightly larger in the disturbed sites for both models (Table 1., FIG 6).

267 There were no differences in home range size between sexes (Females $\bar{x}=659.32 \pm 149.05$; Males
268 $\bar{x}=337.78 \pm 168.15$. Kruskal-Wallis $X^2_{(1,23)}=1.450$, $P=0.2284$). The lack of data for the dry
269 season did not allow to make statistical comparisons between seasons, although, home range sizes
270 between seasons for both methods were visually smaller during the dry season (FIG 6).

271

272

DISCUSSION

273

274

275

276 **Movement patterns**

277

278 Animal movement is one of the main factors involved in their survival, driven mainly by decisions
279 to meet their primary needs for food, shelter and reproduction but that also exposing them to risks
280 such as predation or, in the case of amphibians desiccation (Henrique, 2017; Semlitsch, 2008;
281 Spiegel et al., 2017; Tucker et al., 2018). We observed that habitat perturbation changes the way
282 in which individuals of the species *I. spiculatus* move, mainly by increasing the distances that
283 individuals travel. Our results suggest that toads modify their movements due to disturbance, a
284 pattern that has been registered in other organisms. For example, *Pteromys volans*, a squirrel native
285 to Eurasian boreal forest areas, increased the speed and the distances of their movements when
286 coming into contact to urbanized zones, they also found a variation in the size of the home range,
287 which is attributed to sex, reproductive behavior and tree structure (Mäkeläinen et al., 2015). In
288 this study, we were able to find toads at patches of secondary montane cloud forest with constant
289 human access, even when it has been registered that the species only distributes at primary cloud
290 forest (Mendelson, 1997). But to do so, individuals have to make adjustments as increasing the
291 distances moved, changing locations more often (reflected in the differences in activity proportion)
292 and by taking longer distances to readjust positions probably in search of better conditions.
293 However, despite our results show that activity of this species is higher at disturbed sites and we
294 could find them at remaining patches of forest near agricultural fields, individuals never crossed to
295 larger deforested zones such as crops. Similar edge effects have been registered in *Ambystoma*
296 *maculatum* a salamander native from United States and Canada. Individuals of this species were
297 monitored at two breeding ponds, one within continuous forest and the other one at the between
298 forest and grassland. Individuals avoided to cross the edge changing their directions when
299 approaching to grassland zones (Rittenhouse & Semlitsch, 2006). Suggesting that for some species
300 especially those with a biphasic life cycle as *I. spiculatus*, these changes interrupt completely
301 connectivity between habitats turning them more vulnerable to population size reductions.

302 Sex was another aspect that influenced movement patterns in *I. spiculatus*. Females tended to move
303 longer distances than males without being disturbance an aspect that influenced this. We consider
304 that this can be related to physiological factors as females need bigger energy requirements to
305 produce eggs. Females in other amphibian species (*Anaxyrus americanus*) can allocate more than
306 half of their energetic reserves to generate a bigger clutch (Finkler et al., 2014), specially prior to
307 the breeding season as in *Rana temporaria* (Jönsson et al., 2009). We think that female toads need
308 to move more in order to cover their energetic requirements for securing reproduction and bigger
309 clutches.

310

311

312 However we have observed that these patterns are affected by extrinsic conditions such as
313 environmental conditions and in particular by habitat disturbance (Todd et al., 2009; Foley et al.,
314 2005; Amo et al., 2006, 2007). In amphibians their need for constant hydration can restrict their
315 movements to areas of constant humidity (Ficetola & De Bernardi, 2004; Russell et al., 2005b).

316

317 **Morphology and movements**

318

319 Apart from energy needs, morphological characteristics also influences variation in movement. For
320 example, studies based on the dispersion of *Rhinella marina* (a species with similar sexual
321 dimorphism) based on the movement related with the limb size to explain the advance in Australia,
322 showed that the females they have in average bigger movements (>70m per day) than the males
323 (Phillips et al., 2006, 2007). On the same way this patterns has been reported in another amphibian
324 species as; *Rana luteiventris*, *Anaxyrus boreas* and *Ambystoma maculatum*, they showed that the
325 distances vary in relation to the big size the females than the males (Bartelt et al., 2004; Mcdonough
326 & Paton, 2007; Muths, 2003; Pilliod et al., 2002). Thus, we can assume that the variation in the
327 movement patterns by sex of *I. spiculatus* is influenced by physiology, which at the same time
328 influences reproductive behavior. In which possibly due to the differences in size and coloration
329 of the females, the mate selection is given by the males. In some salamanders as *Desmognathus*
330 *ochrophaeus*, where selection depends on males, the larger size of females influences selection and
331 reproductive success. Similar behavior has been reported in *Bufo boreas* and *B. wolongensis* (Liao
332 and Lu, 2009; Licht, 1976; Marco et al., 1998; Verrell, 1995; Wells, 2007). Since it is knowing the
333 season and breeding sites of this specie, increase the monitoring time and guide efforts within
334 reproductive zones will allow us to evaluate in best way their vagility.

335

336 **Habitat use**

337

338 Even if movements are influenced by physiological and morphological factors, they are also related
339 to environmental variations caused by habitat disturbance. Solar incidence and temperature have
340 been reported to increase in fragmented areas devoided of vegetation (such as agricultural zones)
341 (Duellman et al., 2007; Parris, 2004; Peltzer et al., 2006; Saunders et al., 1991; Seebacher & Alford,
342 2002; Valentine & Stock, 2008). In addition, refuge selection might be influenced by
343 environmental factors such as temperature, which within our sites showed differences. As
344 perturbed sites are significantly warmer, toads might need to choose cooler or moister places to
345 refuge or to change refuges more often reflected in the higher activity present at perturbed sites.
346 Furthermore, differences in refuge use might reflect differences in refuge availability due to

347 perturbation. Hollows formed by trees' roots seemed to be more common at conserved sites, this
348 due to the relation between tree age and root size. At perturbed sites logging has caused that most
349 of the present trees are younger individuals without external roots yet. Thus, *I. spiculatus* responds
350 to these changes through differential shelter use. As temperature and humidity were different at
351 both sites, we assume that toads, depending on the site, used these shelters according to their hydric
352 and thermal requirements. Studies with snake species found that in order to find suitable shelters,
353 individuals are able to use thermal signals according to their thermoregulatory needs (Webb et al.,
354 2004).

355 In this same sense, in a study to evaluate the desiccation of *Rhinella marina* in relation to the use
356 of refuges using agar models. It was recorded that there is a differential use of shelters depending
357 on their characteristics and seasonality. For example, during the dry season, toads use the same
358 shelters that, using agar models, showed less water loss in this case burrows and wet grass,
359 compared to other shelters (Schwarzkopf & Alford 1996). For the particular case of *I. spiculatus*,
360 this may resemble the increased recorded use of Burrows and fern patches that may be giving them
361 similar protection against desiccation. On the other hand, a more recent study on the use of shelters
362 with the same species *R. marina*, shows that the vegetation as a refuge was only used during the
363 wet season due to its low humidity retention, they also relate the selection of shelters with
364 temperatures low in dry seasons, as an appropriate way to regulate their temperature (Seebacher &
365 Alford, 2002). Similar to this variation in the use of shelters by seasonality, for *I. spiculatus* we
366 found a similar pattern but in relation to the degree of disturbance between the monitoring sites.

367 Another effect of habitat alteration might be changes in refuge availability and as a consequence
368 increased competition for shelter sites. Competition for shelters has been reported in other
369 ectothermic organisms like *Hoplocephalus bungaroides* and has been considered a cause of their
370 population decline (Hyslop et al., 2009; Webb et al., 2004). Even when we only found one case of
371 shelter occupation by two individuals of different species (*Tripurion spinosus* an arboreal anuran
372 and *I. spiculatus*) we can't make any conclusions of this type. Further studies should evaluate this
373 type of interaction.

374

375 **Home range estimation**

376

377 Contrary to what might be expected, we did not find differences in home range (HR) size between
378 sites, an aspect that could have been influenced by the number of relocations per individual
379 gathered during this project. One caveat about our estimations is that, due to the lack of
380 knowledge in relation to reproductive sites we could not include areas nearby breeding zones.
381 Although, our results show a variation in HR sizes between seasons. Even when the results are only
382 close to significance, we can see that during the dry season the HR is reduced. This might have a
383 relation to water availability during that period, a resource that can turn more limited due to habitat
384 disturbance (Ficetola 2004; Fahrig 2003; Peltzer 2006 and Börger, et al., 2006).

385 In the case of amphibians with a biphasic life cycle, movement between aquatic reproduction sites
386 and terrestrial sites in addition to the high levels of phylopatry reported in some species (e.g, *Bufo*
387 *hemiophrys* and *Salamandra salamandra*), the HR can have great size variations between seasons
388 (Keheller and tester 1969; Wells, 2007; Forester et al., 2006). For example, for *Bufo spinosus* and
389 *B. viridis*, the nuclei of activity (50%) is determined by their resting places while the total HR
390 (95%) by feeding areas. In addition, each area is influenced by shelter and food availability
391 (Indermaru, 2009). It is possible that the seasonal variation in HR of *I. spiculatus* can be explained
392 by relating the core areas (KD 50%) with the shelters' use. For example, we observed that during
393 the dry season individuals showed a single and smaller core area (50%) at the disturbed site; this
394 may be related to lower refuge availability and due to unfavorable environmental conditions, such
395 as high temperatures and low humidity (Ficetola et al., 2004).

396 On the other hand, disturbance can play a role in these differences. For example, *Philander*
397 *frenatus*, an opossum from South America, increased its activity in open areas, however presented
398 smaller HR, in comparison to areas of continuous vegetation, due to low availability of food and
399 habitat and due to the concentration of refuges in smaller areas (Delciellos et al., 2017). So,
400 according to these results, it is possible that the HR (MCP method), of *I. spiculatus*, at the disturbed
401 site are larger because the toads move more and cover a much larger area, but in relation to the
402 areas of greater activity (core areas) these are smaller due to resources' limitation.

403 Another possible explanation for the low variation in the size of our estimates may be related to
404 the breeding season. It has been registered that *Vipera latastei*, a viper belonging to the Iberian
405 Peninsula, keeps a small size HR during all year except on the breeding season, when their HR
406 areas increase about five times more in response to changes in male's dispersal searching for mates
407 (Brito, 2003). In addition, when analyzing post-breeding migrations in females of *Bufo*
408 *americanus*, it was discovered that, when moving away from breeding sites, the females found a
409 refuge in which they remained for up to three days, which in turn also generates multiple core areas
410 of activity (Forester et al., 2006). Based on this, we observe that HR is linked to the dispersal
411 capacities of species that vary in relation to sex (Muths, 2003).

412 Although it was not possible to monitor within the breeding sites of *I. spiculatus*, we consider that,
413 like *B. americanus*, the HR size of the females changes as they move away from their breeding
414 sites. However, it is necessary to carry out studies to discern all these hypotheses. Studies focused
415 on the dispersion of this species according to its diet and food availability would help to better
416 understand its mobility and distribution.

CONCLUSIONS

417
418
419
420 Although the movement of this species is influenced by sex, the results of this study show that
421 habitat disturbance has an effect in terms of the movement patterns of *I. spiculatus*, increasing its
422 distances traveled and altering their habitat use in the most disturbed places.

423 Despite the variation in individual distances moved, the size of the home range did not show
424 significant changes. So, we can propose that, to understand the complexity of animal movement,
425 we must be more meticulous in terms of increasing the amount of spatial data and also complement
426 it with behavioral information such as; diet, reproductive behavior and behavioral elements such
427 as personality. This to get a more accurate picture of the key factors that drive movement.

428 We can propose future work in relation to the data collected in this research and at the same time
429 try to give a more comprehensive approach based on the natural history of this species.
430 Understanding the ecology of movement will help us develop and propose new conservation
431 measures that include this aspect.

432
433

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442

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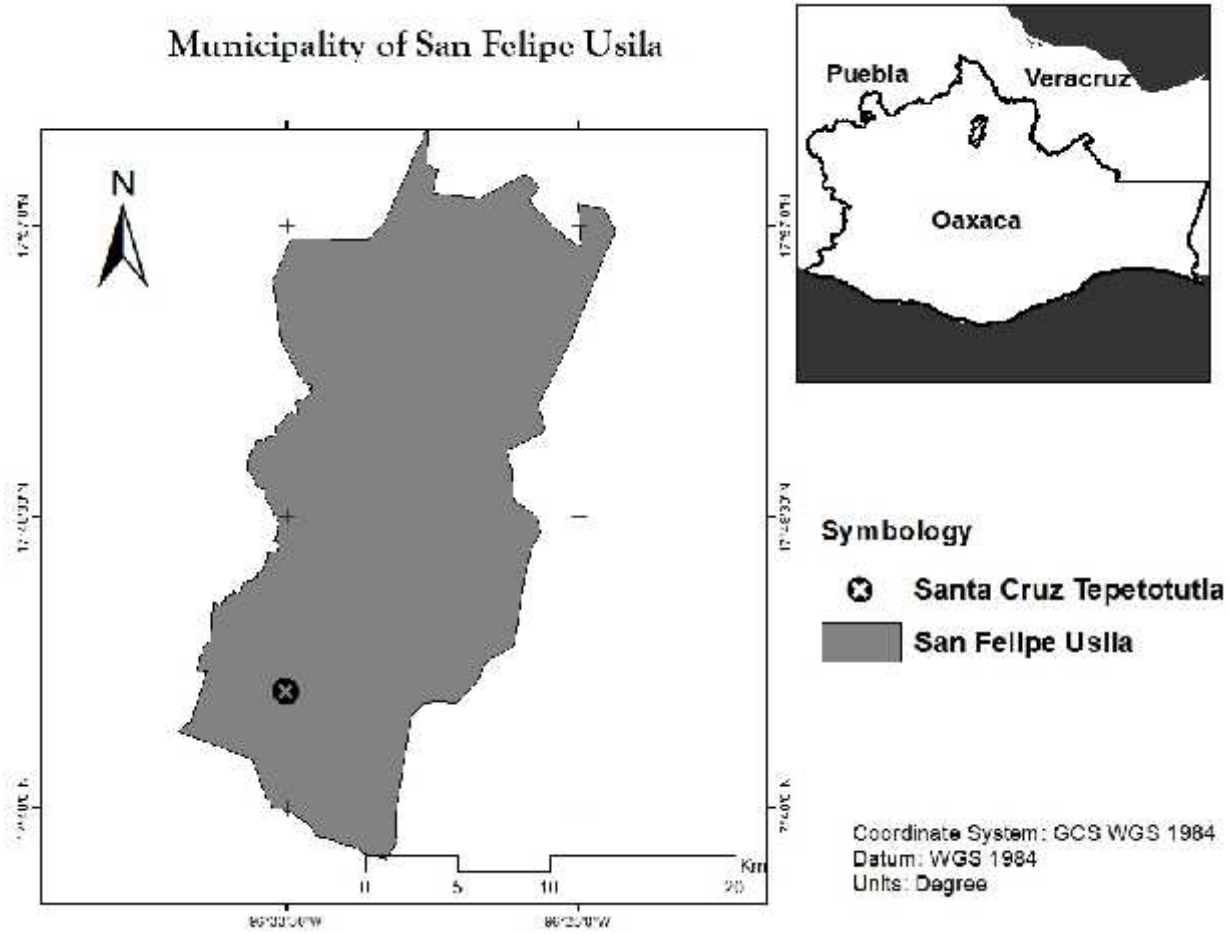
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640 **FIGURE CAPTIONS**

- 641
- 642 FIG 1. Study site. Santa Cruz Tepetotutla Oaxaca. San Felipe Usila Municipality
- 643 FIG 2. Differences in moved distances between sexes in 24-hour cycles $P = 0.01$
- 644 FIG 3. a) Differences between moved distances per site $P = 0.006$. b) Differences between local
645 adjustment distances per site $P=0.009$.
- 646 FIG 4. Differences in distances between seasons $P= 0.054$.
- 647 FIG 5. Shelter use by *Incilius spiculatus* at sites with different perturbation degrees
- 648 FIG 6. Home range estimate by season for both sites a) Conserved site b) Disturbed site. Gray scale
649 polygons represent the MCP method, green outlines represent the total home range (95%),
650 red outlines represent the core areas of activity (50%) using the Fixed Kernel Density.
- 651 Table 1. Conserved and disturbed characteristics according selected sites.
- 652 Table 2. Areas estimated in hectares (Ha) for the home range using MCP and KD, values in bold
653 indicate the largest areas. The disturbed site maintains larger areas than the conserved site
654 for both models except for KD during the dry season.
- 655

656 FIG 1.

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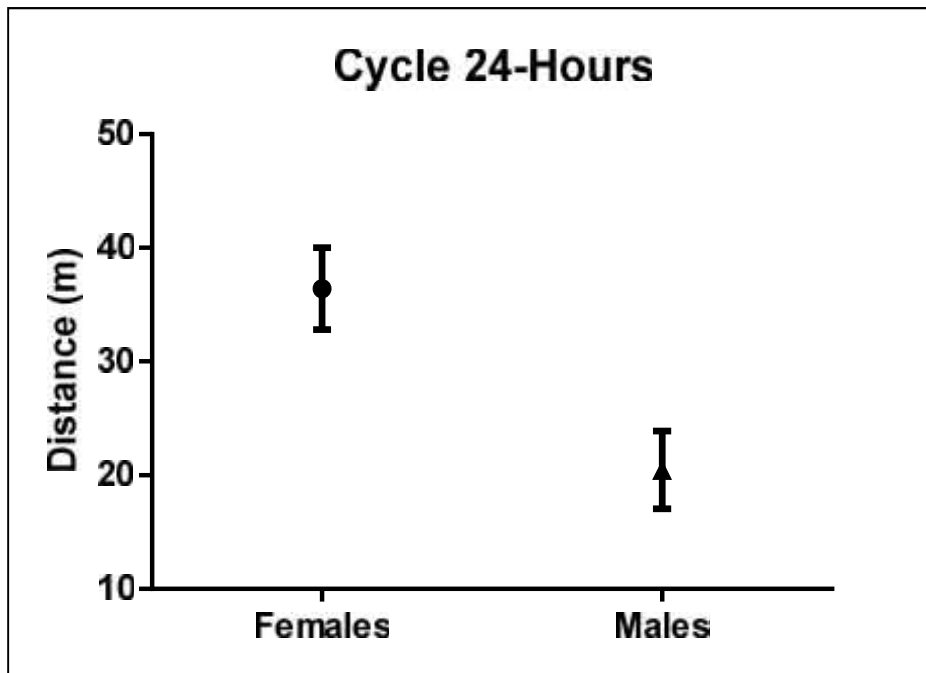
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663 FIG 2.

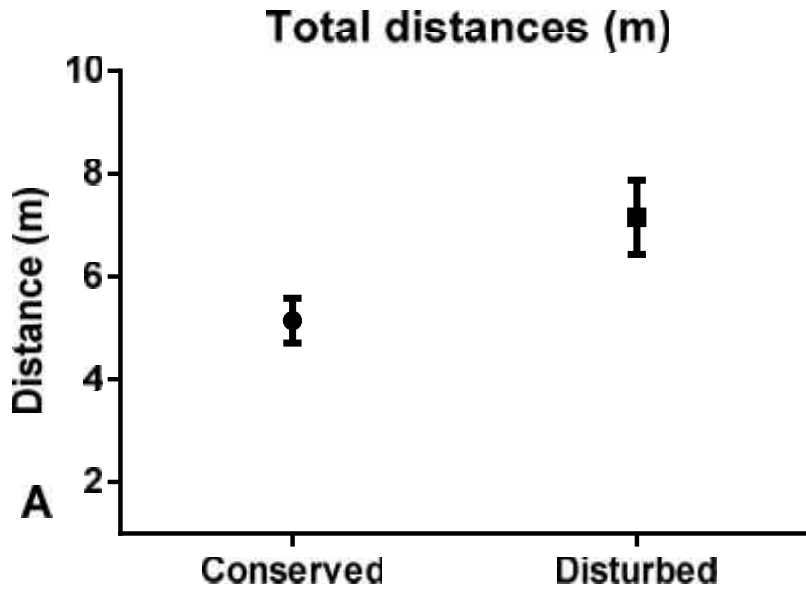
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666 FIG 3.

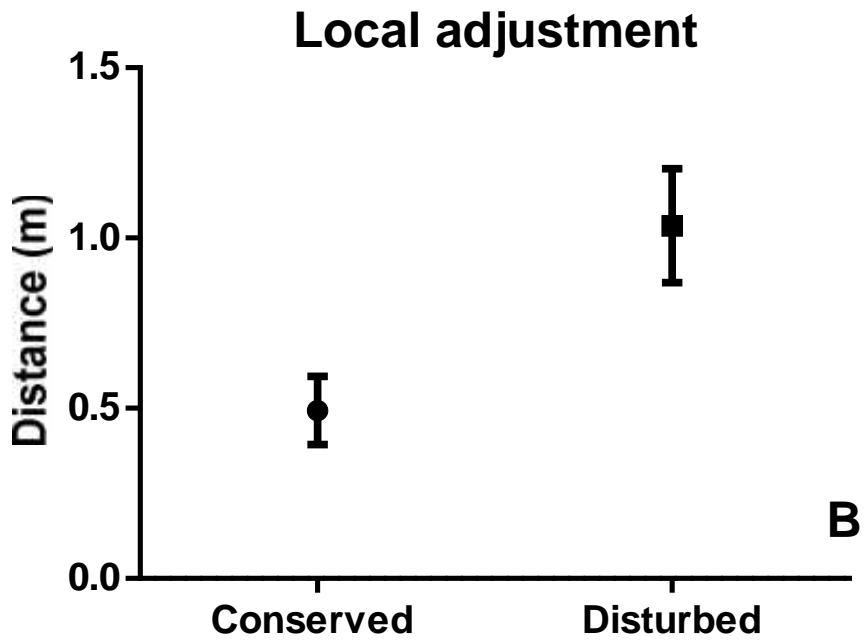
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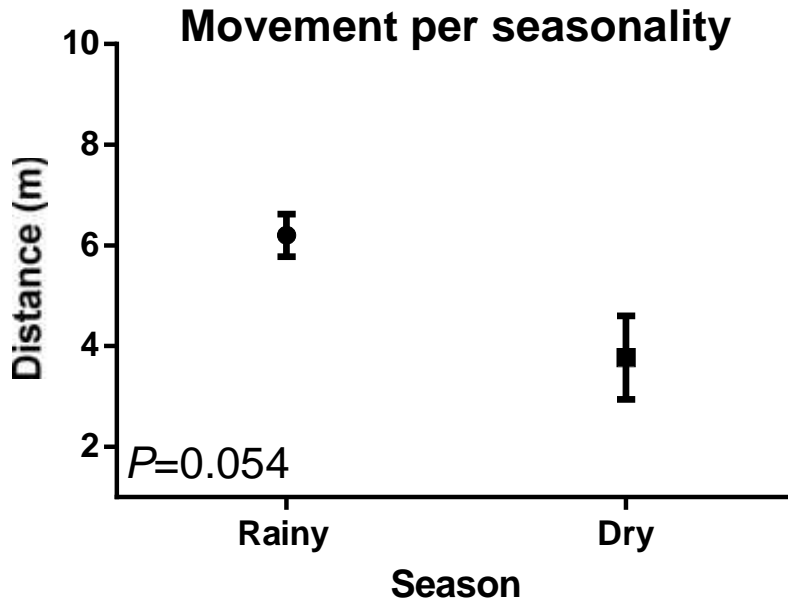


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673 FIG 4.

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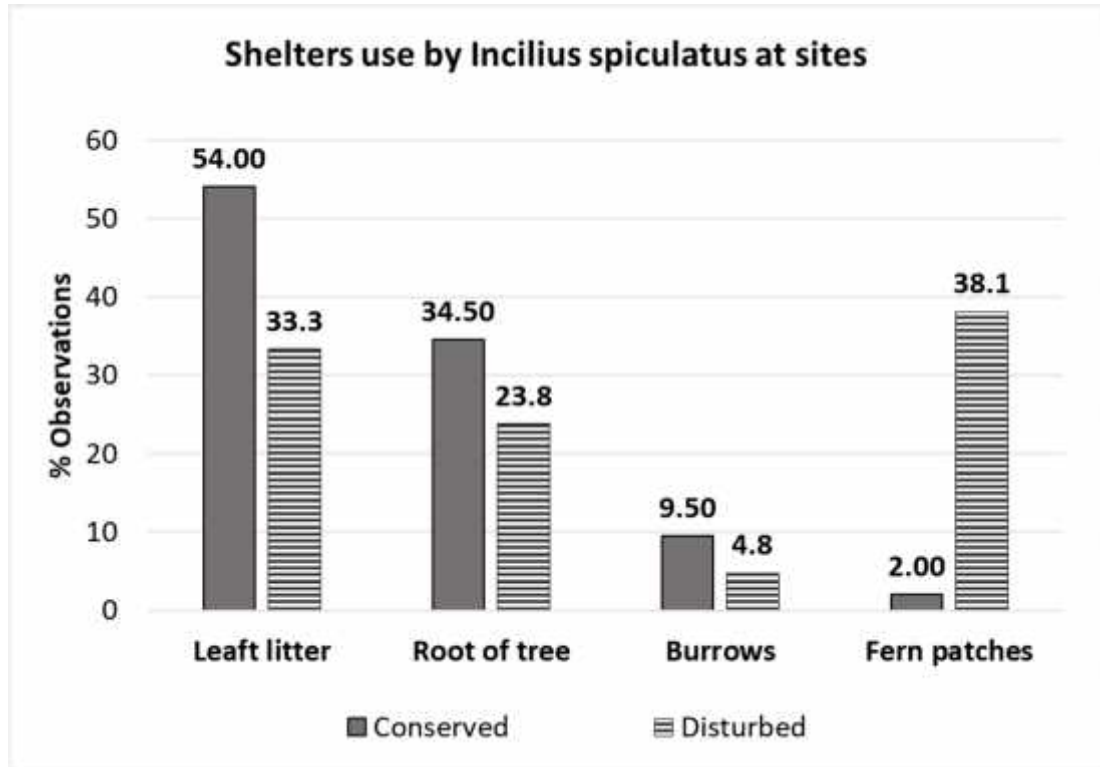
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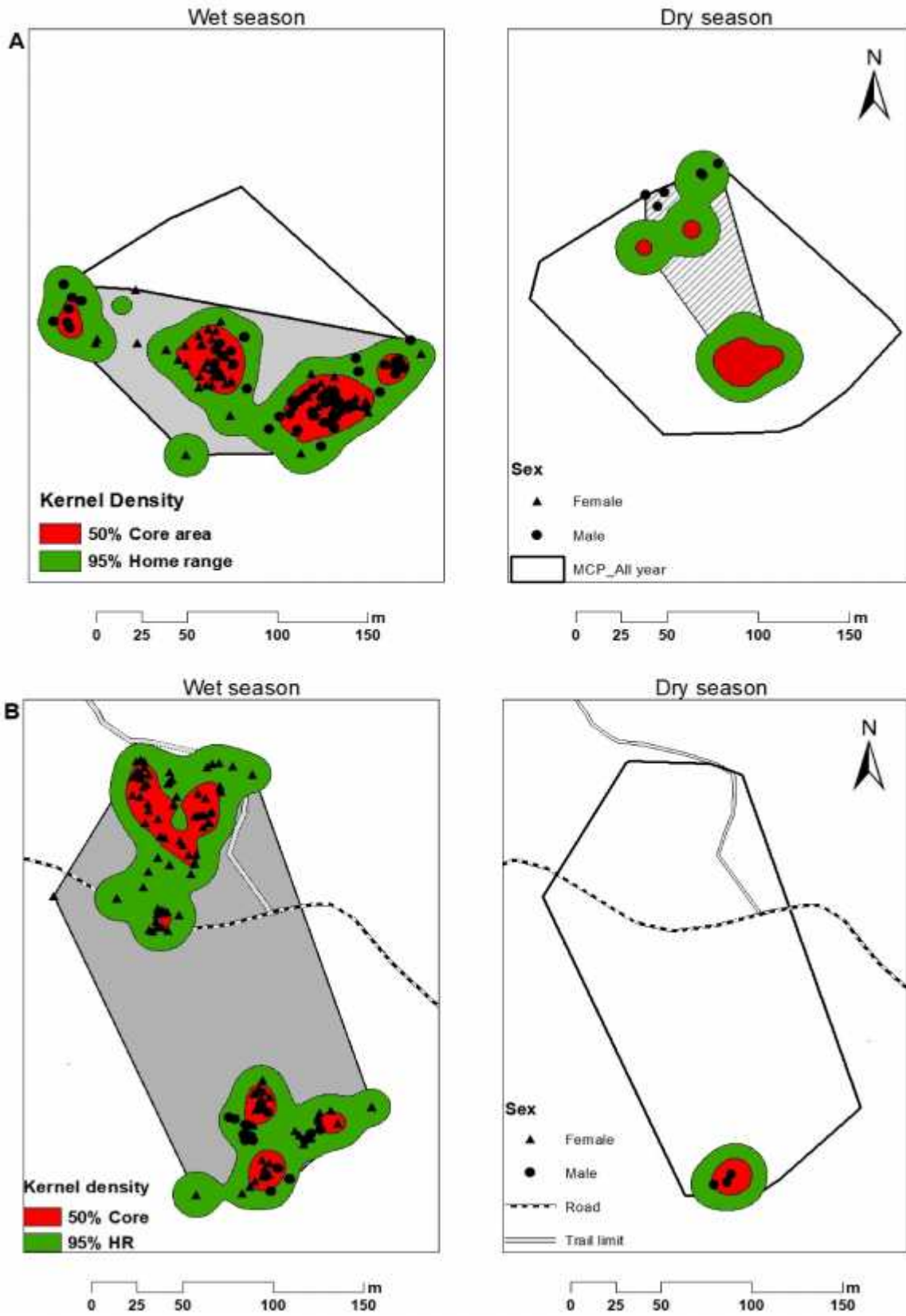
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681 FIG 5.



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685 Table 1

| Monitoring sites | | |
|-----------------------------|------------------------------|--------------------------------|
| Characteristic | Conserved | Disturbed |
| Altitude | 1550 masl | 1300 masl |
| Vegetation type | Primary montane cloud forest | Secondary montane cloud forest |
| Location | Within a conservation area | Within ecotourism areas |
| Wood extraction | No | yes |
| Distance to water bodies | <150m | >500m |
| Distance to dirt roads | Over 1000m. | Aproximately 130m. |
| Maximum ambient temperature | 26°C | 31°C |
| Minimum ambient temperature | 16.5°C | 17°C |

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688 Table 2.

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| Site | MCP 100% (Ha)All year | MCP (Ha)Rainy season | MCP (Ha)Dry season | Rainy season | | Dry season | |
|-----------|--------------------------|----------------------------|--------------------------|----------------|----------------|----------------|----------------|
| | | | | 95% KD (Ha) | 50% KD (Ha) | 95% KD (Ha) | 50% KD (Ha) |
| Conserved | 1.92 | 1.24 | 0.045 | 0.173 | 0.071 | 0.174 | 0.087 |
| Disturbed | 2.68 | 2.168 | 0.01 | 0.453 | 0.021 | 0.089 | 0.036 |

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CAPITULO II

Description of the tadpole and natural history of *Incilius spiculatus* (Mendelson 1997), an endangered toad endemic to the Sierra Madre de Oaxaca, Mexico.

Descripción del renacuajo e historia natural de *Incilius spiculatus* (Mendelson 1997), un sapo en peligro endémico de la Sierra Madre de Oaxaca, México.

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Description of the tadpole and natural history of *Incilius spiculatus* (Mendelson 1997), an endangered toad endemic to the Sierra Madre de Oaxaca, Mexico.

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55

56 **Abstract**

57

58 Amphibian population declines are occurring rapidly around the world. Paradoxically, new
59 amphibian species keep being discovered, reflecting a still growing state of knowledge of this
60 group. A parallel situation is the lack of information that exists regarding life cycles, in particular
61 of those species that have an indirect development with a free-living larval stage. Many amphibian
62 larvae are still unknown or undescribed, thus impeding the proper understanding of the biology
63 and habitat use of a species. In this paper we describe the tadpole of the bufonid anuran *Incilius*
64 *spiculatus*. We also offer a description of the amplexus observed in nature, aspects of its natural
65 history in its adult stage and a forest clade tadpole identification key. This information contributes
66 to the understanding of its life history and its distributional patterns which contributes toward a
67 basis to inform conservation considerations for this endangered species.

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70 **Keywords:** Anuran, forest toads, valliceps group, amplexus, stream breeding, scorpions eaters,
71 diet.

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80 **INTRODUCTION**

81

82 Amphibians are the vertebrates currently at higher extinction risk (Beebee and Griffith 2005;
83 Scheele et al. 2019; Stuart et al. 2008). In recent years, the research on this group has increased
84 exponentially which has promoted the discovery of new species even in highly studied areas
85 (Canseco-Márquez et al. 2017; Jiménez-Arcos et al. 2019). This situation reflects the state of
86 knowledge of this taxon is lower than expected, highlighting the fact that the understanding of the
87 group should be increased in the aim of reducing the risks of losing it.

88 A parallel situation is the lack of information that exists regarding life cycles, in particular of those
89 species that present an indirect development with free-living larval stages. These species usually
90 inhabit different sites across their life cycle and play different ecological roles in relation to their
91 development stage, for example, most tadpoles contribute to keeping healthy water bodies by
92 consuming algae and breaking down organic material (Cortés-Gomez et al. 2015), while
93 transferring energy between aquatic and terrestrial habitats (Flecker et al. 1999; Kupferberg 1997).
94 As adults, these organisms usually prey on invertebrates, thus controlling their populations and at
95 the same time, acting as prey for other organisms (Duellman and Trueb 1994; Stewart and
96 Woolbright 1996). This duality also means that both life stages are exposed to different risk factors,
97 so in the aim of conserving species with biphasic life cycles we should increase the knowledge on
98 both stages.

99 Although the discovery of tadpoles from Latin America is increasing, there still are many
100 neotropical species whose larval stage is unknown (Downie et al. 2015; Kaplan and Heimes 2015;
101 Köhler et al. 2015). During recent surveys in the northern slopes of Sierra de Juarez in Oaxaca, we
102 found an amplexant pair of *I. spiculatus*. This species was described from adult specimens
103 deposited in scientific collections but no tadpoles and breeding behaviour of the species were
104 recorded nor described despite the extensive fieldwork that has been made in the region (Caldwell
105 1974; Lips et al. 2004; Mendelson 1997).

106 In this paper we describe the tadpole of *I. spiculatus* in addition to information on breeding sites,
107 amplexus type and clutch size. We provide an identification key to the forest toad's (Mendelson et
108 al. 2011) tadpoles and observations on the adult stage with comments on conservation implications
109 that might allow this endangered toad to persist in the region.

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114 **MATERIALS AND METHODS**

115

116 We conducted surveys at San Pedro Yolox (17.589359°N, -96.551790°W) datum WGS84 and
117 Santa Cruz Tepetotutla (17.739446°N, -96.558292°W), located on the northern slopes of the Sierra
118 Juárez, Oaxaca, México, within the sub-physiographic province Sierra Madre de Oaxaca (Ortiz-
119 Pérez et al. 2004).

120 In February 2019 we found a pair of *I. spiculatus* in amplexus at Rio Coyul, San Pedro Yolox
121 (17.64015°N, -96.4306°W, 645 m asl). A total of 70 eggs were collected and taken to the
122 Amphibian Conservation Ecology Laboratory at CIIDIR-Oaxaca. Eggs were kept in glass tanks
123 with aerated water. The water was replaced every two weeks and tadpoles were fed with boiled
124 spinach and lettuce *ad libitum*. The room temperature ranged from 23 to 30°C. Tadpoles were
125 euthanized with 5% lidocaine and preserved in 10% formalin.

126 A total of 34 tadpoles at Stage 26 to 37 (Gosner 1960), were examined using a microscope (Carl
127 Zeiss 2000-C), and photographed (Canon Powershot GX5) for their subsequent measurements. For
128 tadpole morphology, we followed terminology of Altig (1970, 2007). Measurements were made
129 with tpsUtil and tpsdig2 software (Rohlf 2017, 2019). Photographs of the oral apparatus were taken
130 specifically at Gosner stages 21 and 27. The oral formula followed Altig and McDiarmid (1999).
131 Live coloration and codes were described following Köhler (2012). All series were deposited in
132 the Museo de Zoología Facultad de Ciencias (MZFC #) at the Universidad Nacional Autónoma de
133 México.

134 Larval development time under laboratory conditions was measured by recording the progress
135 between the 46 Gosner stages until full metamorphosis was reached.

136 **Clutch size**

137 The approximate total number of eggs in the clutch was calculated by multiplying the average
138 number of eggs in 10 cm (counted at five different sections) by the total measured length. The
139 total length is approximate as we did not want to affect the clutch. In addition, air and water
140 temperature were taken and the river width measured with a flexometer.

141 **Adult diet**

142 We recovered fecal samples from six adult toads from Santa Cruz Tepetotutla in order to examine
143 items in the diet. The samples were preserved in 70% alcohol and were examined with a microscope
144 (Leica model EZ4 stereoscopic). Food items or structures were identified with specialized insect
145 keys (Palacios-Vargas et al. 2014; Ríos-Casanova 2014; Vélez and Vivallo 2018).

146 RESULTS

147 Tadpole description

148 Average measurements (mm) for Gosner Stage 27: body length 6.08, tail length 9.15, tail muscle
149 height 1.53, maximum tail height 2.84, total length 15.23, tail muscle width 1.10, internarial
150 distance 0.92, interorbital distance 1.79 Measurements for other Gosner stages are presented in
151 Table 1. The body is ovoid in dorsal view, widest at about middle point and narrower near the tail;
152 expressed in lateral view. Snout nearly semicircular in dorsal profile, rounded at the tip in lateral
153 profile. Spiracle sinistral with inner wall free from body. External nares ovoid, situated nearer to
154 eyes than to snout. Eyes dorsal. Vent tube medial. Tail rounded at the tip. Caudal musculature
155 highest at base, gradually tapering to a pointed tip; dorsal fin reticulated.

156 Oral disk small; labial tooth row formula 2(2)/3, A1 slightly longer than other rows; A2 gap
157 narrow, approximately width of 3-8 teeth' P1 and P2 equal in length, P3 is the longest posterior
158 row; labial papillae incomplete dorsally and ventrally, disposed in two interposed lines on the
159 lateral area of the jaw (Fig. 1 A). At Stage 21 the larvae show an elongation of the oral apparatus
160 that protrudes and separates from the body (Fig. 2 D-E).

161 In life, the color of the body is uniformly brown cinnamon 43 (Kohler 2012), except for the ventral
162 part of the mouth where the color becomes paler. The venter is slightly transparent, with counter-
163 clockwise coiled intestine visible. Around Gosner Stage 35, small cream yellow 82 (Kohler 2012)
164 dots appear throughout the body and the iris. The tail fin is transparent with large pigment granules
165 forming a reticulate pattern on the dorsal fin. Around the Gosner Stage 37, the ventral part of the
166 limbs are pale brown cinnamon with cream yellow dots and dark brown bars dorsally. In
167 preservative, the tadpole body and the tail musculature present natal brown 49 (Kohler 2012), while
168 the ventral part of the body is slightly translucent.

169 Tadpole development took approximately 35 days to complete metamorphosis under laboratory
170 conditions. Three days after their collection (8 February), embryonic development reached Gosner
171 Stage 12. Three days later they reached Stage 18 and four days later Stage 25.

172 Adults

173 Breeding behaviour

174 *Incilius spiculatus* uses the shallower margins of rivers to reproduce, where the water current is
175 slowed by the presence of rocks and aquatic vegetation. "Río Coyul" is a permanent river at 640
176 m asl, with an average width of 8.19 m at the site where the amplexus was recorded (Fig. 2 F).

177 The toads use the vegetation and the material on the bottom of the river to maintain their position
178 in the water during amplexus. Mating was observed during the day and the amplexus is axillary
179 (Fig. 2 A-B). At the time of our observations (12.50 h GST) the water temperature was 19.6 °C.

180 The approximate clutch size was of 4556 eggs and it was attached to the aquatic vegetation at the
181 river margins at 35.2 cm depth (Fig. 2 C). A second clutch was observed at another pool located
182 3.70 m away. We observed amplexant behaviour for approximately 2 hours and 40 minutes, from
183 the time of encounter until the toads separated. The female body temperature was 21.0°C; SVL
184 85.5 mm and clutch temperature was 21.0 °C. In addition to the couple, a second male toad inside
185 the water was observed. Reproduction occurred during the dry season.

186 **Diet**

187 From the examined fecal samples, we found that Hymenoptera were the most important prey (15
188 individuals/ 48.3%) followed by Coleoptera (7 individuals/ 22.5%), scorpions (6 individuals/
189 19.3%), Orthoptera (2 individuals / 6.4%) and Blattodea (1 individual / 3.2%). Based on field
190 observations, *I. spiculatus* is opportunistic and largely insectivorous in its feeding habits. The
191 feeding strategy presented by this species is sit and wait.

192 **Extension of elevational range**

193 Previously, the distribution range reported for *I. spiculatus* was from 800 to 1689 m asl (Mendelson
194 1997). We observed two juvenile individuals at Santa Cruz Tepetotutla, Oaxaca, in a patch of
195 primary cloud forest vegetation at an elevation of an altitude of 1758 m asl (17.71862°N, -
196 96.55911°W) datum WGS84. We also recorded three individuals in San Pedro Yolox at 682, 643,
197 642 m asl (17.63622°N, -96.42735°W, 17.64001°N, -96.43061°W, 17.64013°N, -96.43056°W,
198 respectively). With these records, the altitudinal range of this species is extended to 642-1,758 m
199 asl.

200 **DISCUSSION**

201 *I. spiculatus* is an endemic species of the southeast of Mexico, restricted to highlands of the
202 physiographic subprovince of Sierra Madre de Oaxaca (Mendelson 1997; Ortiz-Pérez et al. 2004).
203 It occurs mainly in montane cloud forest and is endangered (EN) according to the IUCN (IUCN
204 2020; Mendelson 1997). Even though this area has been highly explored, (Caldwell 1974;
205 Caviedes-Solis et al. 2015; Delia et al. 2013; Lips et al. 2004) the larval stage of this species was
206 unknown (Mendelson 1997). With this description, the number of known tadpoles for the forest
207 toad's group is increased to eight, only missing to describe *I. melanochlorus*, and *I. campbelli* (Altig
208 1970; Korky and Webb, 1973; Mendelson et al. 1999, 2011; Segura-Solis and Bolaños 2009;
209 Shannon and Werler 1955). Among the tadpoles of this group, *I. tutelarius*, *I. macrocristatus*, *I.*
210 *leucomyos* and *I. spiculatus*, in addition to *I. valliceps* share the oral formula 2 (2) / 3 (Korky and
211 Webb 1973; Limbaugh and Volpe 1957; McCraine and David 2000; Mendelson et al. 1999). Even
212 when *I. valliceps* is not a forest toad, we decided to include it in the identification key as it is
213 sympatric with *I. spiculatus* and could be confused with it due to its similar morphology. The
214 difference for *I. spiculatus* is that A2 gap is of 3–8 teeth wide and that it presents a reticulated
215 pattern only on its dorsal fin.

217 A peculiar morphological characteristic that *I. spiculatus* presents in Gosner Stage 21 and
218 disappears in Gosner Stage 25 is an oral apparatus that protrudes from the body (Fig. 2 D-E). As
219 far as we know this feature has not been reported for other species. A limiting factor to contrast it
220 with other species is that most descriptions are often made from tadpoles in developmental stages
221 beyond Gosner Stage 25, so larval development in earlier stages is generally unknown. The closest
222 related species for which we could find a complete larval development description (from
223 fertilization to metamorphosis) is *I. valliceps* and it does not have this mouth type (Limbaugh and
224 Volpe 1957). As mouth shape is associated both to habitat type and diet in other amphibians (Altig
225 and McDiarmid 1999; Van-Buskirk 2009) we consider this structure might be a foraging adaptation
226 in early development that switches later in this species. Diet type at this stage should be investigated
227 to elucidate its function.

228 In relation to breeding behaviour, our findings confirm that *I. spiculatus* uses lotic systems during
229 the dry season to reproduce, a consistent pattern among the forest toad group (Mendelson et al.
230 1999). With this new data, the information on reproduction sites for the nine species of this group
231 is now completed (Mendelson 2011). Reproduction in lotic environments is a rare behavior in
232 amphibians however, it might be related to the dry season as is the moment when these systems
233 present slower currents and are shallower. In this way, eggs are not dragged while waterbody
234 desiccation does not represent a risk for the larvae (Kam et al. 1998; Wells 2007).

235 Even when the sample size for adult diet analysis was small, we obtained the first description of
236 the diet of this species, which is composed of arthropods, including ants, beetles, crickets and
237 scorpions. Probably the most noteworthy observation is the evidence of scorpion consumption,
238 which is poorly documented in amphibians. Basically, in the neotropics, all the reports of scorpions
239 in the diet occur in three families (Bufonidae, Hylidae and Leptodactylidae) and seven species:
240 *Leptodactylus pentadactylus*, *Leptodactylus bolivianus*, *Leptodactylus fuscus*, *Osteopilus*
241 *septentrionalis*, *Hypsiboas pugnax*, *Bufo (Peltaphryne) peltocephalus*, *Rhinella marina* (Botero-
242 Trujillo 2006; Flórez and Banco-Torres 2010). With this observation, information on scorpion
243 predation within the bufonidae family is increased.

244 A broader dietary analysis should be taken in order to compare possible differences between ages
245 and sexes in the aim of determining different roles in the ecosystem as proposed for other toad
246 species. For example, preferred prey size is related with body size and age in *I. cristatus* which as
247 juveniles consume smaller prey than adults, while females, bigger in size, consume bigger items.
248 Thus, suggesting possible different predator-prey interactions according to their age (Gelover et al.
249 2001; Oropeza-Sánchez et al. 2018).

250 Despite the fact that the elevational distribution of this species is now extended from 642 m asl to
251 1758 m asl, potential reproductive streams are located at lower elevation (642 m asl in this study).
252 As lowlands present warmer climate, fertile soil and less slope they are usually attractive for
253 agriculture and human settlements, which results in clearing and deterioration of the vegetation

254 cover, thus contributing to connectivity loss between living and breeding sites (Price and Butt 2000;
255 Velazco-Murguía et al. 2014). This has different implications for the conservation of the species.
256 First, reproductive adults and post metamorphic juveniles emerging from the river are forced to do
257 migrations through disturbed sites from and towards forested areas increasing desiccation risk
258 (Oropeza-Sánchez et al. 2018; Todd et al. 2009; Walston and Mullin 2008). While tadpoles
259 developing at the river get higher exposure to chemicals used in agriculture and other human
260 activities like sand and water extraction (Adlassnig et al. 2013; Sparling et al. 2001).

261 These aspects are an additive factor to the already vulnerable situation of amphibians, like *I.*
262 *spiculatus*, with narrow distribution areas, aquatic reproduction and larvae development in lotic
263 systems, which turns them more susceptible to habitat transformation increasing their risk
264 extinction probabilities (Nowakowski 2017).

265 Increasing efforts to discover the larval stages of anurans is needed in the aim of completing the
266 knowledge that we have about amphibians at risk. First, because it is necessary to understand their
267 habitat requirements and possible differential stressful factors. Second, because tadpoles remain
268 longer in bodies of water, so they are often the only evidence of the presence of amphibians at the
269 sites, taking relevance for the realization of rapid inventories that allow us to know more accurately
270 the diversity of this group. For this reason, the morphological description of tadpoles allows to
271 detect species that in their adult phases are more complicated to register, while reducing costs and
272 technical complications of inventories that involve molecular techniques such as bar codes
273 (Grosjean et al. 2015).

274 Even under these circumstances and despite the report by IUCN (2020) that this species is not
275 distributed within protected areas, the local community conserves large areas (9,570 ha, in the case
276 of the municipality of San Felipe Usila) of montane cloud forest under the Indigenous and
277 Community Conserved Areas (ICCA's) mechanism. ICCA's are areas governed by indigenous or
278 local communities where collective action focuses on the governance of common resources at
279 multiple scales (Bray et al. 2012). These social actions contribute to the maintenance of optimal
280 habitat where the species can still survive. Communication with the local community to share
281 findings on the reproductive behaviour should increase chances of improving habitat quality at
282 lower land and ensure the survival of this species.

283

284

285

286

287 **Key to the tadpoles of the Forest toads of Mexico and Central America**

288

289 1a. A2 Gap present 4

290 1b. A2 Gap absent..... 2

291

292 2a. Known from Mexico..... 3

293 2b. Tail fin light brown with widely dispersed dark brown dots;

294 know from western Costa Rica and Panama..... *I. aucoinae*

295

296 3a. Known from Sierra Madre Oriental of Veracruz and Puebla, México..... *I. cristatus*

297 3b. Known only from Sierra de los Tuxtlas Veracruz, Mexico..... *I. cavifrons*

298

299 4a. Tail fin transparent 5

300 4b. Tail fin uniformly dark brown *I. tutelarius*

301

302 5a. Tail musculature black 6

303 5b. Tail musculature brown 7

304

305 6a. Tail musculature partially black with scattered pale areas. Known from southern México to western

306 Guatemala..... *I. macrocristatus*; *I. aurarius*

307 6b. Tail musculature black. Tail fins reticulated and flecked with black. A-2 gap width about 2 labial teeth.

308 Known only from northeastern Honduras..... *I. leucomyos*

309

310 7a. Tail musculature uniformly dark brown. Dorsal fin presents large pigment granules forming a reticule.

311 A-2 gap width equal to 3–8 labial teeth; Known only from Sierra Madre de

312 Oaxaca.....*I. spiculatus*

313 7b. Tail musculature brown; dorsal and ventral fins with yellow reticule, A-2 gap wide, width equal to 10–

314 15 labial teeth..... *I. valliceps*

315

316

317

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319

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326

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464 FIGURE CAPTIONS

465

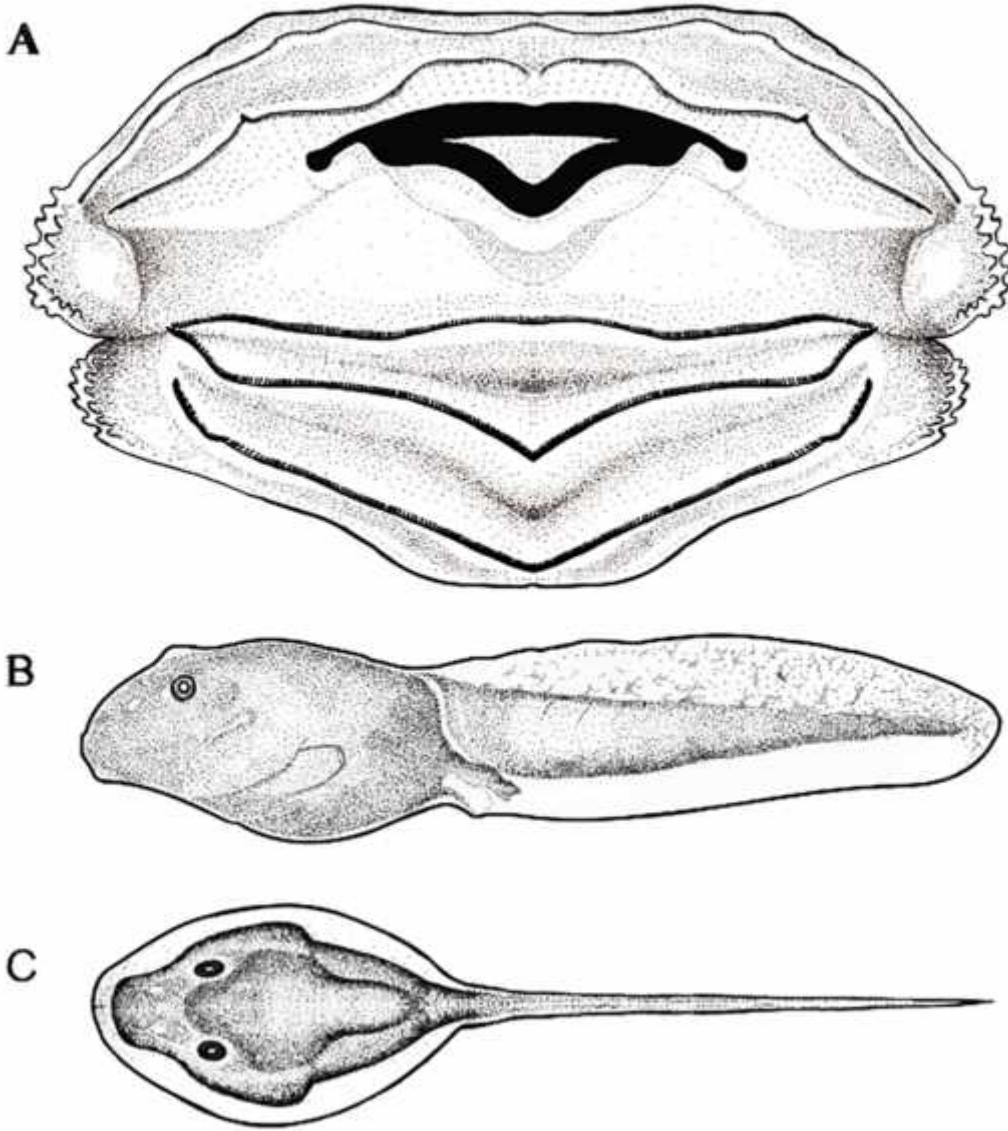
466 Fig. 1. Tadpole of *I. spiculatus* a) oral disc, b) lateral view, c) dorsal view.

467

468 Fig. 2. Breeding behaviour of *I. spiculatus*. A,B) Amplexus (axillary type) and oviposition; C) Clutch; D,E)
469 Dorsal and ventral view of the tadpole head at Gosner Stage 21 showing the “elongated mouth”, F)
470 *Rio Coyul* of San Pedro Yolo; G) Lateral view of tadpole (Stage 39) and H) lateral view of
471 metamorphic individual.

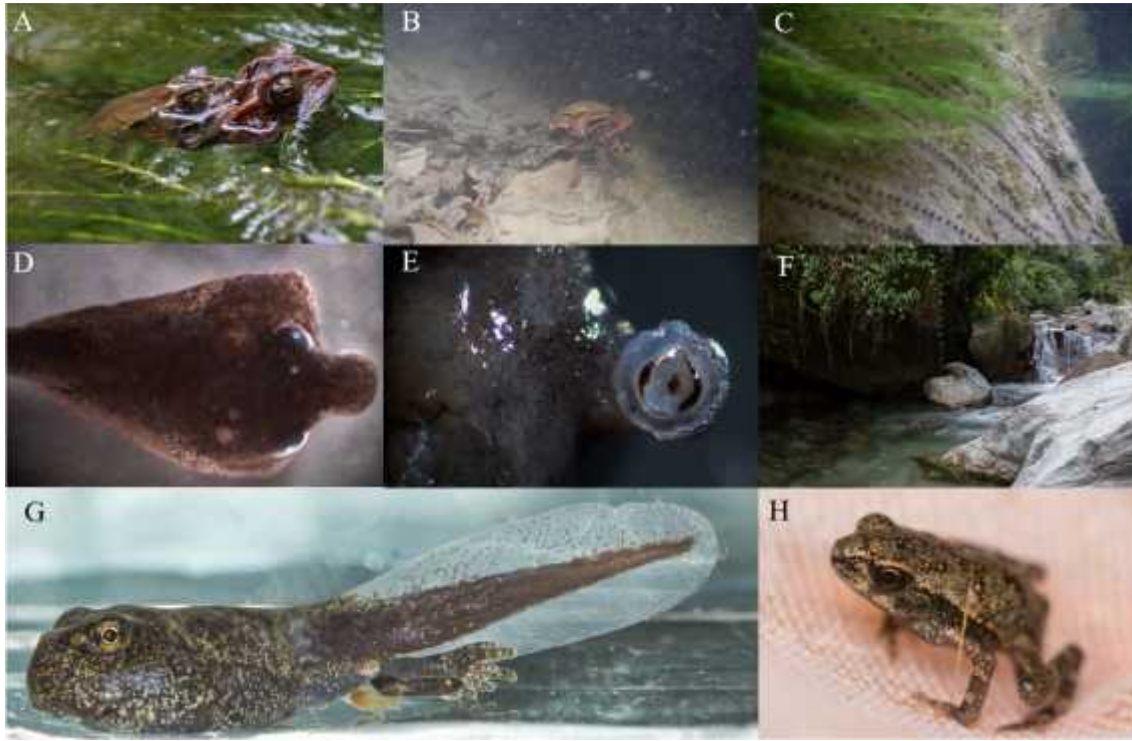
472

473 Table 1. Measurements by Gosner stage (average in mm). Body Length (BL), Tail Length (TAL), Tail
474 Muscle Height (TMH), Maximum Tail Height (MTH), Total Length (TL), Tail Muscle Width
475 (TMW), Internarial Distance (ID), Interorbital Distance (IOD)



506 Fig. 2.

507



508

509

510

511

512 Table 1.

| Stage | Lateral Wiew | | | | | Dorsal View | | |
|-------|--------------|-------|------|------|-------|-------------|------|------|
| | BL | TAL | TMH | MTH | TL | TMW | ID | IOD |
| 26 | 6.25 | 9.87 | 1.38 | 2.85 | 16.12 | 1.00 | 0.92 | 1.87 |
| 27 | 6.08 | 9.15 | 1.53 | 2.84 | 15.23 | 1.10 | 0.92 | 1.79 |
| 28 | 6.61 | 9.84 | 1.52 | 3.22 | 16.45 | 1.27 | 1.15 | 2.25 |
| 29 | 8.04 | 11.20 | 1.82 | 3.91 | 19.25 | 1.38 | 1.15 | 2.26 |
| 30 | 8.95 | 12.12 | 1.95 | 4.26 | 21.07 | 1.55 | 1.26 | 2.57 |
| 31 | 8.63 | 11.77 | 1.75 | 4.16 | 20.40 | 1.45 | 1.28 | 2.50 |
| 32 | 8.51 | 12.80 | 2.12 | 4.38 | 21.31 | 1.52 | 1.32 | 2.49 |
| 33 | 9.03 | 13.61 | 2.74 | 4.63 | 22.63 | 1.68 | 1.28 | 2.69 |
| 34 | 8.79 | 13.60 | 2.14 | 4.59 | 22.39 | 1.66 | 1.32 | 2.62 |
| 35 | 13.28 | 21.68 | 3.41 | 6.09 | 34.96 | 3.31 | 1.66 | 4.70 |
| 36 | 12.89 | 21.76 | 3.29 | 5.90 | 34.66 | 2.81 | 1.59 | 4.12 |
| 37 | 13.90 | 22.73 | 3.44 | 6.29 | 36.63 | 3.05 | 1.70 | 4.84 |

513