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Two other new species of *Rana* (ANURA: RANIDAE) in the Mexican Pacific coasts of Guerrero and Sinaloa.

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RESUMEN

Recientemente, se han vinculado el fenotipo y el genotipo de varias poblaciones del género *Rana* que se habían confundido con la especie *R. forreri* Boulenger, 1887. Aquí se describen otras dos especies nuevas *R. adleri* y *R. hillisi* que se distribuyen, una al noroeste y otra al sur de la Planicie Costera del Pacífico de México. Al parecer, en el país, ambas especies posiblemente estén en estado de conservación estable, por lo menos en las áreas oficialmente decretadas como protegidas.

Palabras clave: *Rana forreri* especie válida, *R.adleri, R. hillisi*, especies nuevas de ranas leopardo, Guerrero, Sinaloa, México.

ABSTRACT

The two new species described here: *R. adleri, R. hillisi*, both range throughout the western and southern Mexican regions, on the hillsides and coastal plains of the Pacific. It was determined that there is congruence between the morphological and the preexisting genomic data of both species. Likewise, and in agreement with what other authors have previously mentioned, many species of the same genus were found waiting to be described based on the same data used here, as well as some other ecological and phylogeographic data. Apparently, the two new species are in a stable conservation state due to the possibility that they are mainly distributed within officially protected areas.

Key words: *Rana forreri* valid species, *R. adleri, R. hillisi*, new species of Leopard frog, Guerrero, Sinaloa, México.

INTRODUCTION

Several authors suspect that the valid species *R. forreri* contains populations that, up to now, could not have been morphologically differentiated, but that are sequenced or electrophoretically identified, as in the cases of other well-known species such as the ones from Central and South America and others from México (Hillis *et al*. 1983). In addition, some congeners numbered as 1-8 (Hillis and Wilcox, 2005 or 1-30 Zaldivar-Riverón *et al*. 2004) are possibly new species, like the two recent found species just described (Pérez-Ramos and Luja-Molina, 2022). A population of the genus was reported in the region of Acapulco (Platz, 1991). It is unknown if it corresponds to a micro-endemic species, which was associated to an unmistakable species, previously and broadly well-known, and it was suggested to be a population of *R. berlandieri*. Later, it was also suggested that such population was associated to the *Rana sp*. Papagayo form (Zaldivar-Riverón *et al*. 2004).

Considering the latest proposed phylogeny for Mexican Pacific frogs (Zaldivar *et al*. 2004), taking a sample of Leopard frogs into account, and considering the intra- and interspecific variability of the Mexican species of the *Rana* genus (*sensu lato* AmphibiaWeb, 2020; Yuan *et al*. 2016), it has made it possible to distinguish another two new species. In México, there are twenty-nine described frog species belonging to the genus *Rana* -used here in a conservative way-. Among these species there are big and small frogs, as well as the enormous introduced species, *Rana catesbeiana*. While the last one has been recently known to be already present in most Mexican states (Pérez-Ramos, unpublished data), the former species are distributed in both coastal plains (Atlantic and Pacific), as well as in the mountainous systems, rivers, basins and interior lakes of the Mexican territory, respectively. Most of the species (75%) are endemic to México; however, it seems there are several species (more than a dozen, pers. obs.) not yet described.

The six phylogenies carried out until now (Hillis *et al*. 1983; Zaldivar-Riverón *et al*. 2004; Hillis and Wilcox, 2005; Che *et al*. 2007; Wiens *et al*. 2009; Pyron and Wiens, 2011), which include some species of genus *Rana*, differ in their results (typological tree). These phylogenies are here taken with caution because some frogs are more conspicuous than others, and some species are apparently more difficult to distinguish. In addition, there is a lot of information about some of these species, but little or null data on others. Likewise, it is need consensus on the phylogenetic methods used.

Until now, except for Hillis *et al*. 1983, *R. forreri* was believed to have a wide distribution along the Pacific coastal plains (PCP), from Sonora to Chiapas and maybe to Central America. In an attempt to establish the phylogenetic relationships among the species that are distributed in the slope and coast of the Pacific, some populations were molecularly differentiated, but their taxonomic status was left unresolved (Zaldivar-Riverón *et al*. 2004).

The Pacific Coast Clade (PCC) defined by Hillis *et al*. 1983 contains, among others, the species of *Rana* that are mainly distributed in or along the PCP, from Sonora to Chiapas Some of these populations are already sequenced and electrophoretically identified,

but not morphologically differentiated, while others are phenotypically but not genetically characterized, so their taxonomic status is incomplete. Subsequently, the observations presented here were corroborated with the clades' terminal species found by other authors. This suggested the concordance between the morphological data used here and previous phylogenetic hypothesis (Hillis *et al*. 1983; Zaldivar-Riverón *et al*. 2004; Hillis and Wilcox, 2005).

Using several recent specimens from different localities, situated always to the south of the Pacific coast plain in México –first those from Laguna de Tres Palos, then those from other adjacent localities, and finally those from states as distant to the northwest as Sonora, Sinaloa, Nayarit, Jalisco, and even more distant states up to the southern region of Chiapas (near the international limits between México and Guatemala) –, it was possible to differentiate and describe two frog species that are distributed partially within this coastal plain. As for Guatemala, one of the species described here is perhaps item of its amphibian fauna, but sampling of the coastal zone of both countries is needed to verify its taxonomic status and establish its phylogenetic relationships.

The aim of this study is to describe two new species of Leopard frogs from the Mexican Pacific versant, which are allocated to the paraphyletic genus *Rana* (*fide* Yuan *et al*. 2016).

METHODS

Through an *in vitro* examination of over 408 specimens, as well as through the assessment of photographs and some live specimens, it was possible to differentiate several frog populations of the *Rana* that are related to others populations from the Pacific coast, two in Pérez-Ramos and Luja Molina (2022) and two more described, here. These populations are easily distinguished by their coloration pattern and their morphology, and they were molecularly supported previously (Zaldivar-Riverón *et al*. 2004; Hillis and Wilcox, 2005). This analysis suggested the possible occurrence of diagnosable and distinguishable populations within the apparently wide-ranging species *Rana forreri* Boulenger, 1883 (*sensu lato*) and its congeners.

The descriptions follow what has been suggested by several authors (Zweifel, 1954; Frost and Bagnara, 1976; Hillis and Frost, 1985; Webb, 1988). The dorsolateral folds (DLF) classification (Pace, 1974) is used here. Most of the assessed specimens are fixed in 10% buffered formalin solution and preserved in 70% ethanol.

Since several of the assessed specimens were donated, they were not cataloged in the moment in which this study was conducted, so they were listed with their original field numbers. These specimens will be deposited in the scientific collection of the Science Faculty, UNAM, as soon as possible.

RESULTS

Taxonomic Account

These observations, in agreement with previous phylogenetic studies, suggest the following hypothesis: since both congeners of the PCC have not been named, but have

already been sequentially differentiated (Zaldivar-Riverón *et al*. 2004; Hillis and Wilcox, 2005) and morphologically characterized here, it is suggested to name both species.

It is suggested that first species is named as:

Rana adleri new species
Mayo Leopard Frog (suggested English common name)
Rana leopardo mayo (suggested Spanish common name)
Fig. 1 (right); Table 1, Appendix 1

Rana forreri Boulenger, 1883 (in part)
Rana forreri 1 Zaldivar-Riverón et al. 2004

Holotype.— MZFC 22763 adult male México, Sinaloa, Mpio. Culiacán, Dren Proto, 24.802104, -107.509150, ca. 14 m (georeferenced).

Allotype.— MZFC 5994 adult female México, Sonora (*sic*), Mpio. Guasave, zona de Guasave, poblado Juan José Ríos, canal de riego secundario 25.757222, -108.821667, 10 m (The state should be Sinaloa instead of Sonora INAFED, 1986).

Paratypes.— MZFC 16707 adult male Mpio. Guasave, sistema de riego 63 de Guasave, cerca de río Yaqui, canal de riego, 39 m; MZFC 22761 adult male, MZFC 22764-22768 adult females, same data as the holotype; MZFC 22769-22771 adult males, MZFC 22772 adult female, MZFC 22773 adult male, MZFC 22774 adult female México, Sinaloa, Mpio. Culiacán, Canal "La Plancha", sistema de riego Ojo Culiacán-Humaxa (*sic*) 24.937056, -107.418083, 60 m (Contact zone).

Haplotype.— MZFC [14186] adult male, México, Sonora, Mpio. Huatabampo, 1 km S Estación Don, sobre carretera Mex 15 (entre Estación Don y Miguel Hidalgo) 26º21'37.2", 109º0'59.7", 161 m (see note on haplotype below) (Contact zone).

Topotype.— MZFC 5994-2 adult female, same data as Allotype.

Diagnosis
The large species *Rana adleri* is distinguished genotipically and phenotipically from its congeners in its coloration, morphology, as well as in its geographic distribution. In a simple way, by means of the following combination of characters: from the 27 well-known species (in addition to the *R. hillisi*, also described here; as well as of *R. cora*, and *R. floresi*, recently described), it possesses a robust body and a thick head (wider rather than longer), dorsoventrally compressed; in possessing large squared-dark-marks pattern or frequently paired-dark-marks pattern on the anterior dorsum surface vs. medium or small dark-marks at random pattern on the back in all species; in presenting fragmented dark transverse bands among the transversal dark and light bands, but the fragmented bands can be located either in the distal, middle or proximal areas of the dorsal surface of the tibia-fibulars; in that in the same surface, the bands that are not fragmented tend to have a pendulum shape or, occasionally, an "L" shape. In the remaining species, it is common to observe the light and dark

transversal alternate fringes on the hind limbs (except for *R. cora*, *R. floresi*, and *R. hillisi* see Table 1).

Description of holotype

Adult male, with snout-vent of 99.88 mm; head length 32.63; head width 36.57; femur length 43.73; tibia length 51.65; naris-orbit 9.07; eye diameter 8.34; tympanum diameter 7.97. Continuous, wide, thick, heightened DLF. Smooth skin on belly, granulated on the cloacal surface. In preservation, light brown background on the belly and dark brown on the dorsum, apparently squared-dark-mark pattern or frequently paired-dark-mark pattern on the anterior dorsum; immaculate belly, except for the conspicuous dark and light reticulated pattern along the mandibular surface, whitish supralabial thick line; light brown eardrum membrane with a whitish central point. The back with sixteen enormous dark brown marks, most of them oblong, sometimes few elongated or rounded, with a whitish green halo; lateral surface with brown marks, typically large but a few smaller than those on the back; these marks are apparently longitudinally aligned in a paired disposition; four femoral dark bands (the three proximal ones are wide bands) on the dorsal surface of the hind limbs; posterior surface of the thighs markedly reticulated with large light and dark marks; dorsal surface of the forelimbs with a big dark square mark (the cubito-ulnar mark). Short webbing of hind limbs, but it extends laterally until the blunt tip of each finger. Proximal rounded subarticular tubercles, while the distal is somewhat enlarged; also an enlarged metatarsal tubercle but of medium size.

Variation in the type series

The specimen MZFC 22762 shows exceptionally big eyes and a dorsal pattern of small and numerous stains on the back and lateral surface (diagnostic characters of *R. magnaocularis*); but it concurs in having a wide head, dorsoventrally compressed, and a dorsal pattern of the hind limbs with marks similar to those in *R. adleri*. There is also a variation in the femoral pattern: although it presents big femoral marks, it has five bands in the left hind limb, but four in the right one. In addition, both DLF are displaced, so such specimen could be considered a *R. adleri*X *R. magnaocularis* hybrid.
On the other hand, the haplotype MZFC 14186 was sequenced (Zaldivar-Riverón et al. 2004); however it morphologically represents a hybrid specimen between *R. forreri*X*R. adleri*, which entails, for both species, taxonomic and systematic difficulties which surpass the scope of this study. The haplotype possesses the typical tibia-fibular marks of the *R. adleri* but not the typical head and body proportions, nor the pattern of marks on the back and on both sides. Although enlarged, these marks do not reach the conspicuous dimensions of those in *R. adleri*. Likewise, for its distribution, it corresponds to a specimen with characters of at least two species, as it usually occurs in contact zones.

In the type series, there is a gradual variation in the diagnostic characters: on the dorsal tibia-fibular surface and between the dark transversal fringes, the light areas may vary from one to three, since the dark areas are fragmented or may even present mixed conditions of light bands and dark L-shaped areas such as those present in the paratype (MZFC 16707) or, given the case, to be pendulum-shaped marks as in the topotype (MZFC 5494-2). Likewise, in comparison, both specimens have a more robust

body conformation than the rest of the specimens of the type series. Nevertheless, all the individuals of such series present the diagnostic characters of the nominal species: at least one light area present among the dark fragmented transversal fringes on the dorsal tibia-fibular surface; there are a few large dark marks on the back and on the four limbs. Other complementary characters are shown in Table 1.

The specimens MZFC 22773 and 22774 of the type series are here considered as hybrid forms between *R. adleri* X *R. cora*, because both present dark cuneiform tibia-fibular marks (character property of the later species), few enormous dark marks on the body (character property of the former species); and a stout body, typical of *R. adleri*.

In the type series, the diagnostic characters of the pattern of dark marks on the body and legs changes also as follow: 12-18 big dark marks on dorsum; 3-7 dorsolaterals dark marks; 3-5 femoral dark marks: 1-3 big transversal tibia-fibular dark marks that may be fragmented, therefore leaving light areas of the same hue as that of the dorsal background of the legs; 1-3 cubito-ulnar dark marks, but at least one of them big. The dark transversal tibia-fibular marks have a pendulum shape in MZFC 22763 (holotype), MZFC 22764, 22765, 22770 (paratypes) and, occasionally, an "L" shape in MZFC 16707 (paratype); additionally, among those dark marks, there are immaculate spaces, the same color as that of the background of the dorsal surface of the hind limbs. Likewise, all specimens, without exception, possess a notable reticulated pattern of large dark and light marks on the posterior surface of the thighs.

Etymology
The species name is in the nominative case and it honors Kraig K. Adler, who inspired our own trips after those he made to Sierra Madre del Sur in the beginning of 1964. He has been our foreign academic advisor and he has promoted the development of herpetology in general internationally.

Distribution
Rana adleri ranges of north to south from the sates of Sonora and Sinaloa in México, into the Mayo= Yoreme region in the PCP northwest region of México, where the natives live.

Ecological Notes
Rana adleri inhabits the irrigation canals from hill and plain regions of the Pacific Coastal Plain within an elevation between 10 to 65 m. In recent time, it has been found mainly in agricultural regions (Figs. 3a and 3b).

Type locality description
Northwest of México, one of the main agricultural regions for the production of legumes and others vegetables, located between the states of Sonora and Sinaloa (Fiscal et al. 2017), within the irrigation system that sustains these agricultural fields and that has seemingly promoted the exploitation of frogs of the genus *Rana*. So far, four species of Leopard frogs are sympatric: *R. adleri*, *R. cora*, *R. forreri* and *R. magnaocularis*. The region is mainly a coastal plain (Figs. 3a and 3b).

Figure 1. Left: *Rana forreri* dorsal view of Holotype BMNH 1882.12.5.7 adult female, SVL= 69.9 mm (Courtesy of J. W. Streicher). Right: *Rana adleri* dorsal view of Holotype MZFC 22763 adult male, SVL= 99.88 mm.

And also here, it is proposed that the second species is named as:

Rana hillisi new species
Acapulco Leopard Frog (suggested English common name)
Rana leopardo acapulqueña (suggested Spanish common name)
Fig. 2 (left); Table 1; Appendix 1

Rana forreri Boulenger, 1883 (in part)
Rana sp. 18 Hillis et al. 1983 (in part)
Rana forreri 6 Zaldivar-Riverón et al. 2004

Holotype.— MZFC 14683 adult female (protogyny), Mpio. Acapulco de Juárez, Carretera Aeropuerto-Acapulco, 16º45'22'' N, 99º45'06'' W, ca. 10 m (georeferenced).
Allotype.— MZFC 14678 adult male, same data as holotype.
Paratypes.— MZFC 1765, 1766 adult females, Mpio. Acapulco de Juárez, Laguna de Tres Palos; MZFC 12832 adult female, Mpio. Acapulco de Juárez, San Pedro Las Playas, 16º49'24'' N, 99º43'54'' W, 100 m (*sic*); MZFC 14017 adult male, Mpio. Acapulco de Juárez, San Pedro Las Playas, aproximadamente 25 km SE Acapulco, 16º49'28'' N, 99º45'59'' W; MZFC 14682 adult female, same data as holotype.
Haplotype.— MZFC [14198] adult female, Mpio. Acapulco de Juárez, Carretera Aeropuerto-Acapulco, Laguna Tres Palos (Zaldivar-Riverón et al. 2004).
Topotype.—MZFC 15953 adult male, 1.9 km W Ejido on Hwy 200, at intersection of Hwy 95 and Hwy 200, 19 km W Acapulco, 16º 58.07' N, 99º 59.5'' W, 18 msnm; collected by J. J. Wiens (JJW 1052).

Diagnosis
The small species *R. hillisi* differs from its congeners in coloration, morphology, and genotype, as well as in its geographical distribution. In a simple way, by the following combination of characters: from the 27 well-known species (in addition to the *R.adleri* described here, and also the recently described species *R. cora* and *R. floresi*) in the frequent possession of longitudinal fringes on the hind limbs or, when appropriate, in the separation or fragmentation of the transverse dark bands on the tibia-fibular surface, which is notably visible in this species with its line or fringe on the hind limbs.

In the other species it is common to observe alternately dark and light cross bands on all limbs (except for *R.adleri, R. cora* and *R. floresi*, see Table 1).

Description of the holotype

Adult female (in protogyny condition) with a snout-vent length of 57.29 mm; head length 21.08; head width 17.7; femur length 29.58; tibia length 33.25; naris-orbit 5.54; eye diameter 6.54; tympanum diameter 4.46; dorsolateral continuous folds and small longitudinal folds on the dorsum; short webbing of hind limbs, which extends laterally to the tip of each digit. Smooth skin on the belly, granular on the cloacal surface. The background color (in preservation) of the ventral surface is light brown, and the dorsal surface is light brown-grayish. Immaculate belly, except for the dark and light indented patterns along the mandible surface; narrow whitish supralabial stripe; continuous narrow white DLF; light brown tympanum with a distinctive whitish spot. Dorsum with 14 distinctive dark brown spots (variable in shape, sometimes enlarged or rounded) with a light green-whitish halo; numerous brown spots on the sides, typically smaller than those of the dorsum, apparently, longitudinally aligned in two lines; four dark femoral bands, three dark wide proximal femoral bands, the other bands on the upper surfaces of hind limbs are narrow; immaculate dorsal area on hind limbs, with dark enlarged marks along the tibia-fibular surfaces; dorsal surfaces of the forelimbs with a longitudinally enlarged dark mark; posterior surfaces of the thighs distinctively reticulated, with enlarged dark marks; one brown longitudinally enlarged mark (or cubito-ulnar mark) on the light brown upper surfaces of the forelimbs.

Variation in the type series

There is a gradual variation in this species within the type series, since one of the diagnostic characters refers to the possession of a tibia-fibular fringe or line but in this case, two different extremes are observed: a continuous fringe or line on the tibia-fibular surface (holotype), and a fragmented one, in which each fragment is always longitudinally enlarged. Likewise, a combination of small squared stains, and at least some longitudinally enlarged stains (topotype) could be present. This last specimen also presents a more robust constitution than that of the rest of the series type. All individuals present the same diagnostic character: the dorsal light area on the tibia-fibular surfaces. The specimen MZFC 10255 seems a hybrid of *R. hillisi* X *R. floresi*, because it presents enlarged marks on the tibia-fibular surface and on the cubito-radial surface (both characters of *R. hillisi*), but its femoral markings are narrow and vary from four to five in number (*R. floresi* characters). The other characters in the rest of the species assessed here that differ from *R. hillisi* are shown in the Comparative Table 1.

Etymology

Patronymic, named after David M. Hillis, who knows ranids better than anyone, and who taught the author to recognize the taxonomic and systematic complexity of the splendid world of the true frogs.

Distribution

Rana hillisi inhabits a coastal range from north to south which includes the states of Guerrero, Oaxaca and Chiapas; Michoacán (perhaps another new species). It could also be a Guatemalan resident (identification through Internet photography).

Ecological Notes

Rana hillisi inhabits lenthic and lothic water bodies of the hillside and the plain regions of the Pacific coastal plain. It is distributed from the sea level to an elevation of 525 m. It occurs in primary vegetation such as tropical deciduous forest, tropical semideciduous forest, tulars, mangroves, marshes, halophylus vegetation, aquatic vegetation, and associations of coastal dunes or disturbed areas (see below).

Type locality description

In the vicinity of Laguna de Tres Palos in Guerrero (Figs. 4a, 4b), in the southeast region of Acapulco, which is covered by native and disturbed vegetation, mainly tropical deciduous forest, tropical semideciduous forest, tulars, mangroves, marshes, halophylus vegetation, aquatic vegetation, and associations of coastal dunes; the elevation varies from sea level to ca. 100 m (Diego-Pérez and Lozada-Pérez, 1994). This lagoon is a seasonally variable saltwater reservoir 1.21-4.57 0/00 (Guzmán, 1987), with an area of 50 km^2, situated between Papagayo and La Sabana rivers which are separated by a 2 km meander that flows into the Pacific Ocean (Yáñez-Arancibia, 1977)

Figure 2. Left: *Rana hillisi*, adult female. Right: *Rana sp.* Papagayo form (*fide* Hillis, 1983), adult male. Both are sympatric species (Courtesy Virginia León Règagnon).

Figure 3a. Type locality of *Rana adleri*, in the basin of Río Culiacán, W Culiacán City, Sinaloa, México. Google Maps. Accessed: December 30, 2019.

Figure 3b. Above: Panoramic views of Juan José Ríos region; Below: Distrito de Riego 063, both in the Municipality Guasave, Sinaloa, México (http://www.riegosydrenaje.com.mx/geomembrana/experiencias-en-el-geomembranizado-de-canales-en-el-distrito-de-riego-063-en-guasave-sinaloa/), and Google Maps. Accessed: Diciembre 14, 2019). Contact zone of four species of Leopard frog: *R. adleri, R. cora, R. forreri*, and *R. magnaocularis*.

Figure 4a. Type locality of *Rana hillisi,* panoramic views of Laguna de Tres Palos ca.10 msnm, Municipality of Acapulco de Juárez, Guerrero, México. (Courtesy of Fernando Abraham Morillo Fernández, March 28, 2018).

Figure 4b. Two panoramic views of the type locality of *Rana hillisi*. Left Laguna de Tres Palos ca. 10 msnm, Guerrero, México. (http://turismomexicoxp.com/guerrero/art-Laguna_de_Tres_Palos_en_Guerrero-887.html). (Accessed: December 19, 2017 ©Mexicoxp.com Guerrero). Right Laguna de Tres Palos ca. 10 msnl, Guerrero, México. (http://mexico.pueblosamerica.com/foto/la-estacion-6). (Accessed: December 19, 2017 ©Fernando Montes de Oca, ©Continental Panoramio).

Rana spp	Dorsolateral folds DLF	DLF extension continuous	Coloration DLF	Membrane between fingers	Femoral dark bands or marks
R. adleri	Yes	Yes	light brown-yellowish	moderate	variable >transversal bands
R. berlandieri[1]	Yes	No	brown-yellowish	moderate	transversal bands
R. forreri[2,7]	Yes	variable[5]	light brown-yellowish	moderate	variable >transversal bands
R. magnaocularis[3]	Yes	No	white-yellowish	short	transversal bands
R. omiltemana[4]	Yes	Yes	light brown	moderate	transversal bands
R. hillisi[7]	Yes	Yes	white	short	variable >marks
*R. floresi**	Yes	Yes	white-yellowish	short	variable >makrs
*R. cora**	Yes	Yes	light brown-yellowish	short	variable >marks

Rana spp	Nº femoral dark bands or marks	Femoral bands	Tibia-fibular dorsal area	Tibia-fibular dark mark fringe
R.adleri	>four	>wide	variable; at least one light area	No
R.berlandieri[1]	four	>narrow	alternate bands	No
R.forreri[2,7]	three or more	wide	>alternate bands[6]	No
R.magnaocularis[3]	four or more	narrow	alternate bands	No
R.omiltemana[4]	four or more	narrow	alternate bands	No
R.hillisi[7]	generally < three	wide	light area	Yes
*R.floresi**	four or more	narrow	variable; light area	occasionally
*R.cora**	>five	narrow	light area	No

Rana spp.	Tibia-fibular dark marks fragmented	Tibia-fibular dark marks shape	Cubito-ulnar dark marks square	Cubito-ulnar dark marks enlarged
R.adleri	ocassionally[6]	pendulum or mixture	Yes	No
R.berlandieri	No	NA	Yes	No
R.forreri	ocassionally[6]	mixture	Yes	No
R.magnaocularis	No	NA	Yes	No
R.omiltemana	No	NA	Yes	No
R.hillisi	Yes[8]	fringe	No	Yes
*R.floresi**	Yes	comma/fringe	Yes	No
*R.cora**	Yes	cuneiform	Yes	No

Rana spp.	Dorsal and lateral dark marks size	Dorsal dark marks pattern	Dorsoventrally compressed head
R.adleri	great	paired anteriorly	Yes
R.berlandieri	medium-small	random	No
R.forreri	large-medium	random	Yes (holotype)
R.magnaocularis	small	random	No
R.omiltemana	small	random	No
R.hillisi	variable (medium-small) frequently lengthened	random	No
*R.floresi**	variable (medium-small) lengthened	random	No
*R.cora**	variable (medium-small)	random	No

Notes. 1. *Rana berlandieri* is assumed to range through the Acapulco region (Platz et al. 1990), but it has been suggested that is related to *R. sp.* Papagayo form (Zaldivar-Riverón et al. 2004). 2. *Rana forreri* seems to present a wide distribution along the Pacific Coast Plain (suggest several authors), this is rejected, here. 3. *Rana magnaocularis* seems to present related populations southward of its known distribution ranges (pers. obs. see Appendix 1). 4. *Rana omiltemana* congener, but the species ranges through Sierra Madre del Sur, in Guerrero. 5. In holotype it is displaced in one side; continuous in the other. 6. See text for character variation, fragments may be present, two as maximum. 7. Protogyny. 8. See text, there is intraspecific variation in the type locality, contact zone. NA: Not apply.*Pérez-Ramos y Luja-Molina, 2022.

Table 1. Comparison of selected characters in eight species of *Rana* genus of the PCP (except for *R. omiltemana*, see Note 4 above), as treated in this study.

DISCUSSION

The genus *Rana* is highly variable (so far it is one of the most diversified genera of anuran amphibians in México), as in morphology as in color pattern. This variation may be reflected genotypically. The genus includes 31 species -plus those that remain to be described- whose genomic studies are additionally necessary to determine their phylogenetic relationships. For the first time, morphological characters are identified, which are diagnostics of each of the species proposed and described recently, two in Pérez-Ramos and Luja-Molina (2022) and two here. Likewise, it is identified that the possibility that the distributions of these species adequately coincides with the Mexican geography, and that there may be a close correspondence with the distribution of previously recognized forms. All the above allow establishing the taxonomic identity of several populations of *Rana*. As to the analysis carried out here, similar results were found for several undescribed PCC frogs of the genus.

This study reaffirms the phenotypic diversity found in previous studies on micro and macro organisms from various regions of the Pacific slopes and the coasts of México. Furthermore, it seems possible that there are similar patterns of inter and intraspecific divergence with respect to phenotypic and genotypic variability in the evolution of frogs of the genus *Rana* (*fide* Parris, 1999), or as in studies previously carried out with other organisms (*fide* Nevo and Beiles, 1989 and references therein), which has been indirectly corroborated.

A morphology-based phylogenetic analysis of *R. forreri* proposed dividing it into multiple species (Hillis et al. 1983), this was corroborated here. Just as it was found in this study that the valid polytypic and widely distributed species *R. forreri* actually represents a complex of species –something that has already been confirmed by other authors (Hillis et al. 1983, and Zaldivar et al. 2004) –, which contains more than twelve populations of easily differentiable morphs. The phylogeny of the haplotypes also suggests that the same species may represent more than one species (Hillis et al. 1983; Zaldivar et al. 2004; Hillis and Wilcox, 2005). This was also corroborated here.

Here it is consider that the high variability in body shape and size and in coloration patterns (size, shape and quantity of dark spots) presented in frogs of the genus *Rana*, particularly in the Leopard frogs, was apparently overlooked or underestimated, depending on the case, and that this could have confused previous authors, who probably believed that they were all the same since, for a long time, they considered it to be of the *R. pipiens* species complex, currently *R. forreri*, from the Pacific coastal plain. This study corroborated the extraordinary diversity of the *Rana* genus.

For example, the paired pattern of huge dorsal spots (which, in part, resembles the checkered pattern of *R. palustris* from the northeastern United States of America and the eastern region of Canada) is prominent in the big *R. adleri*. The unmistakable pattern of tibia-fibular cuneiform spots stands out in the big *R. cora*; while comma-shaped spots and some elongated spots that combine on the same tibia-fibular surface, giving the appearance of L-shaped spots in the small *R. floresi*; finally, the elongated longitudinal marks stand out on the back and on the dorsal surface of both legs in the middle-sized *R. hillisi*. This last character is shared by the allopatric species

R. brownorum, in which it only appears in the elongated dark spots present on the anterior part of the body; another characteristic shared by both species is the presence of a continuous DLF. Likewise, only the transversely elongated dark marks on the posterior surface of the body, mainly of the sacral area are generally shared between the allopatric species *R. berlandieri*, and *R. hillisi*.

The tibia-fibular striped pattern which consists of wide transversal dark marks, is shared by most of the species of the genus *Rana*; while as a shared character among *R. adleri*, *R. cora*, *R. floresi* and *R. hillisi* (some of which are sympatric in several areas) is a variation observed in the transversal dark spots on the dorsal surface of the tibia-fibular region: the spots are divided or they attain extreme conditions such as a longitudinal elongation that frequently covers more than half of the tibia-fibular surface in the last two species; whereas, in the former, the extreme condition is that they are fragmented, either in comma-shaped, wedge-shaped, pendulum, cuneiform shape or even combined.

Another character shared among the same four species refers to the presence of a clear area that may or may not have dark small spots on the dorsal tibia-fibular surface, and that has the same color as that of the general background of the body. However, in *R. adleri*, the dorsal tibia-fibular region shows the beginning of the fragmentation of the transversal bands, which may be in the proximal, middle or distal part of that area, or an overt combination of light areas, fragments, and misshapen marks, with a tendency to elongation (*R. hillisi*) (Figs. 1, 2, respectively) and, given the case, fragmentation along the entire length of the tibia-fibular surface (*R. cora*, and *R. floresi*) (see figures of the latest species in Pérez-Ramos and Luja-Molina, 2022).

Likewise, the four species share the character of scarce membrane among the fingers, since the insertion of the membrane occurs at the level of the first tarsal tubercle of each finger, with a noticing reduction between the fingers 1-2, 2-3, 3 -4.

Therefore, within and among that species of *Rana*, there are variations in body size, shape, quantity, arrangement and shape of the bands present on the femoral, tibia-fibular and cubito-ulnar surfaces, and in the marks present on the entire surface of the body, respectively.

There is sexual dimorphism among the individuals of the species *R. adleri*, *R. cora*, *R. floresi* and *R. hillisi*; in these four species the forelimbs of the males are robust, whereas in the females they are thin. In addition, the light brown vocal sacs in males are widespread and large, and they possess thick thumbs with extensive nuptial pads. The light-colored line in the supralabial fold is another character that distinguishes the sexes. In the females this line reaches the insertion of each forelimb; while in the males it is interrupted at the level of the tympanic membrane. However, it must be emphasized that protogyny is present in these specie, as it is in the specimens MZFC 12835, 14198, 14686, in which there are thick forelimbs, thick thumbs with nuptial pads, no vocal sacs, developing testicles, and a light-colored supralabial line from the tip of the snout to the insertion of the forelimb (indicating that the specimens are females, but with males morphology).

Geography scenery

It is possible that the distribution of these four species (*R. adleri, R. cora, R. floresi* and *R. hillisi*) basically follows the Pacific Basins. Pérez-Ramos and Luja Molina (2022) recognized five main regions and five sub-regions, including the main rivers; so, both authors, identified several contact areas.

Conservation

The conservation status of *R. adleri* has not yet been evaluated. Apparently, it is not distributed in any high-priority area or in any protected natural region. Seemingly, the agricultural environment where it has been found could continue to benefit the frogs and other organisms associated with irrigation canals; however, the optimal usage of the hydrological resource has promoted the tubing of the canals (Fig. 3), and this will possibly put some of these organisms at risk or near to extinction.

Possibly, the only species that is somewhat in any risk of conservation is *R. adleri*, since its type locality corresponds to an urbanized area with a tendency to boundless growth. In addition to this, water resource tends to be economized and used better through the piping of the irrigation canals of this entirely agricultural area (Fig. 3). In areas near to its distribution, the water could be affected by chemical pollutants used in agro-industry, but research is still lacking on the influence of the anthropogenic activities on the frog species and other organisms associated with such environments.

On the other hand, as a popular inhabitant of one of the main national and international tourist centers in México, *R. hillisi* is in serious regional trouble, mainly due to the excessive urban growth and the water resource that it requires for its biology. Both territory and water are monopolized by the people of the renowned port of Acapulco, being economically profitable, consumes everything: air, land, sea, lakes, lagoons and rivers, vegetation, and fauna. Despite everything, *R. hillisi* represents an insignia species in this region, since its presence is a sign of the quality of these bodies of water. The frog's environment has been drastically modified by temporal meteorological processes and by the consequences of recent climate change, as well as by the widely exploited resources required for roads, urbanized areas and other human activities. Despite this, it has recently been noted that several populations of this frog still persist in Tres Palos Lagoon, in nearby areas and in other regions in the southern part of the PCP.

Undesirably, the basic biology (demography, geographic distribution, ecology and evolution, among other topics) of the species has been overlooked. However, there have been important breakthroughs in the knowledge of the entomological fauna associated with the species *R. cf. forreri* (Cabrera-Guzmán et al. 2007) formally *R. hillisi* (in part), which has been described previously. Likewise, it could be in danger of extinction due to its interaction with the people. Paradoxically, its conservation and sustainability are linked to certain human activities, such as real estate developments or recreational areas (places such as golf clubs, Figs. 4a, 4b) and federal areas (airport), which could facilitate the feasibility of proposing an ecological reserve near to the famous port of Acapulco, Guerrero.

However, since both type localities of *R. adleri* and *R. hillisi* are allocated in economically revenue-yielding regions, it is possible that they are in a critical conservation status. For this reason, it is convenient to suggest the early establishment of flora and fauna conservation areas: one near the region of the paradisiacal port of Acapulco, and the other one in the capital of the state of Sinaloa. As has been proven in other regions of the country, these conservation areas are an alternative that gives excellent social, cultural, economic, and ecological results when being used in sustainable eco-tourism.

Likewise, the species *R. floresi* also has more possibilities of being protected, like the other amphibians and reptiles found in the conservation area of the natural reserve of the Chamela Biology Station, UNAM (Reserva de la Biosfera Chamela- Cuixmala), a RAMSAR site on the coast of Jalisco.

For its part, *R. cora* appears to be protected, since it is distributed in the Marismas Nacionales Biosphere Reserve in Nayarit and in a RAMSAR site, both recently decreed as conservation areas in the region occupied by the Cora civilization of Nayarit and its adjacent regions. This is why these four species of frogs, and other associated flora and fauna, may have a chance of surviving in this Anthropocene Era.

Finally, *R. adleri*, *R. cora*, *R. floresi* and *R. hillisi* could possibly be considered as bio-indicators of these regions since they denote the conservation status of the different fresh water bodies in their respective distribution areas. However, in other regions such as Los Chimalapas, between the states of Chiapas, Oaxaca and Veracruz, it is well known that some species of the genus *Rana* can inhabit putrefied waters (personal observations). In México, the studies of the entire genus *Rana* are a pending issue. Some of them are emphasized, such as biology, phylogeography, ecology, population genetics, conservation, systematics and taxonomy. In addition, it is necessary to broaden our knowledge of contact areas such as those mentioned here or those referred by other authors (Yuan *et al*. 2016, in part). More than twelve morphs have been differentiated and hopefully they will be corroborated through further phenotypic, genomic and phylogeographic analyzes.

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Appendix 1

Identification keys to seven species of Leopard frogs of the *Rana* genus from the Pacific summit, slope and coastal plain. (Adult and young specimens, some characters used here only regionally).

1a. Head wider than long and dorso-ventrally compressed; mainly dark enlarged dorsal marks, larger than or equal in size to the tympanum; a few marks on the dorsum (paired pattern in the anterior surface), and dark enlarged marks on the flanks; pendulum dark marks on the tibia-fibular dorsal surface; robust appearance…..... *R. adleri* (Fig. 1 right)
1b. Head variable, wide or slender; mainly dark dorsal variable marks, equal in size or smaller than the tympanum, numerous marks on dorsum and flanks (random patterned); robust or slender appearance..... 2
2a. Dorsolateral continuous folds, at least on one side..... 3
2b. Dorsolateral distally displaced folds…….…….……....….....….....….. 7
3a. Hind limbs with alternated dark and light cross bands; bands usually in a transversal complete pattern on the tibia-fibular dorsal surface..... 6
3b. Hind limbs with variable pattern of dark marks; fragmented pattern on the tibia-fibular dorsal surface and with a light dorsal immaculate area..... 4
4a. Hind limbs with dark marks on the tibia-fibular surface; the marks are small and with a dorsal dark longitudinal stripe pattern..... *R. hillisi* (Fig. 2 left)
4b. Hind limbs with dark marks on the tibia-fibular surface; the marks are large or small, usually cuneiform or with a comma-shaped pattern..... 5
5a. Hind limbs with dark large cuneiform marks pattern, usually on the tibia-fibular dorsal surface..... *R. cora*
5b. Hind limbs with dark small comma-shaped marks on the tibia-fibular dorsal surface; usually combined with a longitudinal stripe..... *R. floresi*
6a. A few dark large oblong dorsal marks (random pattern)…..... *R. forreri* (Fig. 1 left)
6b. Many dark small rounded dorsal marks (random pattern)……..…… *R. omiltemana*
7. Large eyes, larger in diameter than the tympanum; many small dark rounded dorsal marks (random pattern)..... *R. magnaocularis*

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