Revista Mexicana de Biodiversidad 85: 414-428, 2014
DOI: 10.7550/rmb.42015

Geographic variation in *Incilius occidentalis* (Anura: Bufonidae), an endemic toad from Mexico, with a redescription of the species and delimitation of the type locality

Variación geográfica en *Incilius occidentalis* (Anura: Bufonidae), un sapo endémico de México, con una redescripción de la especie y delimitación de la localidad tipo

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Abstract. A taxonomic revision of the populations referable to the wide ranging species *Incilius occidentalis* was conducted based on 10 morphometric variables and other external morphological traits. Thirty three populations were geographically defined from more than 220 locality records of the species. A brief summary of the nomenclatural history of this species is presented and the external morphological variation is analyzed. *Incilius occidentalis* is here redescribed on the basis of comparisons with the 3 specimens housed at the Old Collection of the Torino Museum, and over 850 specimens held at several herpetological collections. Statistical analysis using principal components analysis (PCA) demonstrated that SVL concentrates the main part of the variance observed in these populations. No distinguishable populations were detected on the basis of morphometric differences using the Tukey HSD analysis. Body proportions are fairly similar between the 33 populations defined, suggesting the idea that the typical body shape of the bufonid genera is also perceived in this species. The species *I. occidentalis* is fully redescribed, 1 lectotype and 2 paralectotypes are designated. The geographic range of this species is accurately defined and the type locality is restricted to the City of Guanajuato, and surroundings, Mexico.

Key words: amphibiofauna, morphometrics, taxonomy.

Resumen. En este trabajo se revisó la variación geográfica en la morfometría de las poblaciones que conforman la especie *Incilius occidentalis*, que se distribuye ampliamente en el noroeste y centro de México; se presenta además una revisión de la historia nomenclatural de esta especie. Para analizar la variación morfométrica se definieron 33 poblaciones provenientes de más de 220 localidades, midiéndose diez variables morfométricas estandar y otras de morfología externa en ejemplares adultos. El análisis de componentes principales (ACP) muestra que la variación observada se concentra en la variable LHC, sin embargo, la prueba de Tukey-HSD para analizar diferencias interpoblacionales mostró que no existen poblaciones morfométricamente distinguibles dentro de esta especie. La forma y el tamaño del cuerpo son similares entre las poblaciones, lo que sugiere la idea de que la forma típica que define a los bufónidos puede confirmarse en esta especie. *I. occidentalis* se redescribe mediante la comparación de los 3 ejemplares tipo depositados en la Colección Antigua de la Universidad de Turín, Italia y más de 850 ejemplares depositados en diferentes colecciones herpetológicas. Se designan un lectotipo y 2 paralectotipos, y se define la distribución geográfica de esta especie, restringiendo la localidad tipo en la ciudad de Guanajuato y alrededores.

Palabras clave: anfibiofauna, morfometría, taxonomía.

Introduction

The pine toad, *Incilius occidentalis* is a widespread endemic species from Mexico. It has been reported from more than 220 localities from west-central Durango to the south reaching the Isthmus of Tehuantepec on the Pacific versant, and from the Atlantic Versant of San Luis Potosi to the south reaching central Veracruz and eastern Oaxaca (Fig. 1). Although the species is commonly known as the pine toad, it can be found in a wide variety of habitats and microhabitats, such as oak and pine-oak forests, scrublands, arid and semi-arid areas and even cloud forest, at elevations from 150 to 2 600 m. Basic data from the first collections of this toad are unclear, however it can be speculated (from the collector's notes F. Craveri) that

Recibido: 19 septiembre 2013; aceptado: 22 enero 2014

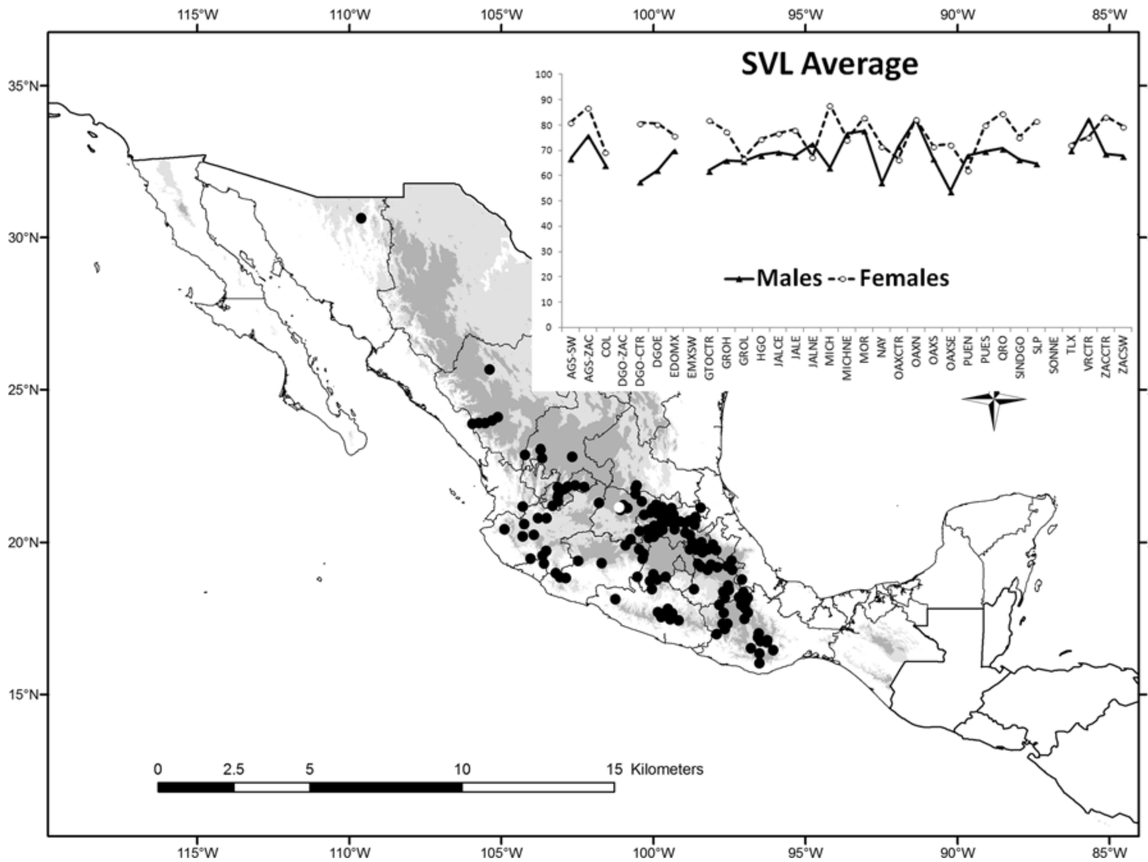

Figura 1. Geographic location and sampling of the 33 populations of *Incilius occidentalis* examined in this study. Numbers refer to populations described in Appendix 1. Snout-vent length average (SVL) for males (black triangles) and females (white circles) can be seen in inset chart.

the 3 specimens in the type series were collected around mid XIX Century. These individuals are housed in the Old Collection of the Torino Museum in Italy. Being a wide ranging species, as well as an ecological generalist, a revision of the taxonomic situation of *I. occidentalis* was needed. The first approach to the taxonomic study of this species was the review of the nomenclatural history, and secondly the analysis of its geographic variation in morphology and morphometry.

Review of the nomenclatural and taxonomic history of Incilius occidentalis. The nomenclatural history of the toad *I. occidentalis* has been poorly discussed although several changes have occurred since its description. In the first half of the XIX century the Italian explorer F. Craveri, collected in Mexico 3 toads which he referred to the genus *Bufo*; he did not record the precise locality or the date of collection. These individuals were donated to the Herpetological Collection of the Torino Museum, Italy (MZUT) and catalogued as *Bufo occidentalis* by De Fillippi (presumably, the collection manager according to the annotations in the original catalogue). Around 1878, Lorenzo di Camerano, a naturalist present at the Torino Museum (MZUT) studied these 3 toads and referred to them, in print, as *Bufo intermedius* Günther, 1859. He presented a brief description of these individuals; however, he included the name *Bufo occidentalis* as a synonym of *B. intermedius* because he found the name in De Fillipi's annotations at the museum records (Camerano, 1879). For many years afterward, the name was largely ignored, with only occasional records cited from Mexico, all of them assigned to *Bufo intermedius* (e. g. Dugès, 1869). In Kellogg's classic paper on amphibians of Mexico at the United States National Museum (USNM), the name *B. intermedius* was considered a junior synonym of *B. simus* Schmidt, 1857, and consequently *B. occidentalis* became a synonym of *B. simus* (Kellogg, 1932). Kellogg did attempt to examine the 3 specimens from the Torino Museum, with no success; apparently the specimens were misplaced or temporally lost at the time of his visit. Concerning *Bufo simus*, this species was described by O. Schmidt (1857) from a series of 9 froglets collected by the botanist J. Von Warsewicz at the Chiriqui River, Bocas del Toro, Panamá. The validity of this species was questioned by Firschein (1950) who noted that the type locality and the localities known for *Bufo simus* in Mexico were remote and suggested that both populations should be considered as different taxa. Firschein (1950) also suggested that the collection data of *B. simus* could be erroneous since there was enough evidence that the celebrated collector J. Von Warszewicz was somewhat careless when documenting collection localities (see Savage, 1970 for other examples from reptiles and amphibian collections). On the basis of these uncertainties and the fact that the specimens collected by Warszewicz were juveniles, Firschein (1950) recommended the use of the name *B. occidentalis* Camerano, 1879 for all the specimens collected in México that had been assigned to *B. simus* Schmidt, 1857. Taylor (1951) supposed the rediscovery of this taxon since he examined an adult individual at the Museum of Zoology (UMMZ-58430) from Boquete, Chiriqui River Province, Panamá; he assigned this individual to *Bufo simus*, based on the absence of *ostia pharyngea* and the development of the supratympatic crests. Taylor discern from Firschein's (1950) position to consider *Bufo simus* as a *nomem dubium*. Savage (1972) examined the syntypes of *B. simus* housed at the British and Wien Museums, and noted that there was no morphological relationship between the specimens collected in Central America and those recorded from Mexico. He concluded that probably the syntypes of *B. simus* could represent the juvenile form of an unidentified species from South America. Recently, De la Riva (2004) made comparisons with other *Bufo* species from South America and concluded that *B. simus* is a synonym of *B. spinulosus* (later *Rhinella spinulosa,* Chaparro et al., 2007).

The other taxon related to the *I. occidentalis* history is *Bufo monksiae* Cope, 1879, a species almost never mentioned in the literature (Santos-Barrera, 1995). It was described based on an immature individual donated by Alfredo Dugés who collected the specimen in 1877 in Guanajuato, Mexico. The type specimen (USNM 9896) was examined by Kellogg (1932) who determined that it represented an immature individual of *B. simus* based of the presence of a mid-dorsal line and an interorbital transverse bar. These and other informative features such as the color of dorsal and ventral surfaces as well as the dorsal distribution of skin granules, and cranial crests are impossible to describe because of the poor state of preservation of the type specimen (Santos-Barrera, pers. obs). *Bufo monksiae* probably belongs to a population of *I. occidentalis* from central Mexico. In 2011, a new species was described from the *I. occidentalis* group, this species inhabits at northwestern Chihuahua and Durango, México and was discovered mainly on the basis of morphological and allozymic evidence (Santos-Barrera and Flores-Villela, 2011; Santos-Barrera et al., in prep.).

From a complete morphological inspection of the 3 specimens of *Incilius occidentalis* at the MZUT and the comparison with the extant populations of toads referable to *Incilius (Bufo) occidentalis*, it is evident that this species represents a complex of cryptic species whose alpha taxonomy is presented here. Previous works mentioned *I. occidentalis* as an inhabitant of most parts of western and central Mexico (Smith and Taylor, 1948). At this time, we know that *I. occidentalis* occurs in several localities at a great variety of habitats from intermediate to high elevations (Fig. 1), and for this reason several individuals of the genus *Incilius* have been erroneously assigned to *I. occidentalis* (Santos-Barrera, 1995). The goals of this study were: *1)*, to analyze the morphological and morphometric variation among populations of *I. occidentalis* in order to determine if this information can be useful to identify cryptic species; *2)*, to describe the geographic variation in morphometrics of this species considering the conservative pattern in morphology of toads, and *3)*, to redescribe the species based on the type series specimens in order to finally define the diagnostic characters that unmistakably lead to the correct identification of specimens and populations representing *I. occidentalis*.

Materials and methods

A total of 470 adult and 371 young individuals of *Incilius* (*Bufo*) *occidentalis* from 25 herpetological collections were examined (Appendix 1). According to the geographic location of the records 33 taxonomic populations were defined along the geographic range of the species (Fig. 1, Appendix 2). Ten standard morphometric variables were recorded for each individual in concordance with Mendelson (1998) and Mendelson et al. (2005): SVL (snout to vent length); HL (head length); HW (head width); TL (tibia length); longest diameter of the tympanum (TYMP); eyelid length (EYELID); parotoid length (PAROTOID); inter-orbital distance (IOD), the distance between the inner edge among both eyes; snout-nostril distance (SND), from tip of the snout to the anterior edge of the nostril; and eye-nostril distance (END), from the anterior border of the eye to the posterior edge of the nostril. Measurements were taken using a digital caliper (0.001 mm). All measurements were made by the same person (GSB) and always with the same caliper to avoid errors from different observers and instruments (Hayek et al., 2001). In addition 5 external morphological features were analyzed: *1)*, dorsal color pattern; *2)*, ventral color pattern; *3)*, shape and distribution of the granulation on the skin; *4)*, development and arrangement of the cranial crests, and *5)*, development of webbing of the foot (webbing formulae). Morphological terminology for tubercles on the fingers and toes follows that of Savage and Villa (1986), the webbing formulae is that defined by Savage and Heyer (1967), and Myers and Duellman (1982), and subsequently modified by Savage and Heyer (1997). Description of coloration of body surfaces follows Smithes's catalogue of colors (Smithe, 1975) (numbers in parenthesis). To test for sexual dimorphism an analysis of variance (ANOVA) was performed previous to start the geographic analysis. Based on these results, sexes were analyzed separately (results not shown). Adult males were defined by external inspection checking for the presence of vocal slits as well as nuptial pads in the first and second fingers, otherwise, a combination of general aspect, body size and lack of males features the individuals were considered as females. For an initial approach to the morphometrics of this species a principal components analyses (PCA) was performed with no transformed variables and based on covariance matrix. The PCA is a tool that standardizes and centralizes data, then the independent variables can be reduced to 1, 2, or more components where variation is concentrated or summarized, this is useful when variables are correlated, as is the case in this study (McKillup, 2012; Rencher, 2002). Scores from the 10 components for each individual were stored in the database and used to check for geographic variation by performing an ANOVA with populations as the independent variable. Only for this analysis, populations with less than 5 samples were excluded, causing the exclusion of 10 male populations (3, 7, 9, 10, 17, 18, 19, 21, 23 and 31) and 8 for females (7, 17, 18, 22, 23, 24, 29 and 30). According to the results of the ANOVA, for the variables where significant differences were revealed, a Tukey HSD test (honestly significant differenced) was applied. This test compares each group mean with every other group mean in a pairwise manner, populations differing from the groups designed with a letter can be considered as different (Quinn and Keough, 2003). All statistical analyses were performed with JMP Academic program (V. 10.0.0. Instituto de Ecología, UNAM) and based on the original, non-transformed values of the measurements for the reason that normality distribution of each variable was previously verified with the Shapiro-Wilk test (Tabachnick and Fidell, 1989; data not shown) and because magnitude orders of measurements are at the same rank.

Results

Morphometric analysis. The ANOVA across sexes revealed size differences between adult males and females in *I. occidentalis* (data not shown). Since all variables resulted significantly different, the statistical analyses were performed for each sex separately. The PCA revealed that variation in morphometrics of *I. occidentalis* is concentrated in 5 variables: SVL, TL, HW, HL, and PAROTOID, although the significance of each variable differs in each sex (Table 1). The first component (PC 1) accounts for 91.4% of the variation in males and 89.6% for females, the variable with the greatest contribution to this component is the SVL with scores of 0.85769 for males and 0.88375 for females, respectively. This is understandable since this variable represents the general body size, then, the second variable becomes more important to define differences between populations, this is TL for males (0.32686) and HW for females (0.27170); the third variable contributing to this component is HW for males (0.27121) and TL for females (0.27139). The fourth contribution is provided by HL for males (0.21591) and females (0.19593), the fifth contribution is the parotoid length with scores of 0.12600 for males, and 0.11558 for females. The rest of the variables have an insignificant contribution in this component (see Table 1). The second component (PC 2) accounts for 3.2 and 3.71 of the variance, in males and females. Scores from PCA indicate that TL and HW are the most important variables in males and females, respectively; however, SVL is negatively correlated with these variables, and in general, the contribution of this component is not comparable with PC 1. A graphic representation of the variables performance can be seen in figure 2. The 2 dimensions plot show the concentration of points, this means that the individuals sampled share a similar body size; longitude and direction of arrows indicate the value of each variable to explain the whole variance, it is evident the great importance of SVL in Component 1 in both sexes, although this variable is more conspicuous in males than in females, the same occurs with the variable TYMP. An interpretation of these results is that females are greater in size but also have a broader head whilst males seem to have a small body size with a small head too, however other variables related with head size as IOD, SND and END have an irrelevant role in the morphometrics of the species; the tympanum, when present, is more conspicuous in males (Fig. 2, see taxonomic comments). Results of the ANOVA across populations using the scores of the PCA show significant differences in the components 1, 3, 4, 6 and 10 in males (see table 2). Regarding females, differences arise in the components 1, 4, and 5 (Table 2). The Tukey HSD test applied to these components indicates that there are no distinguishable populations that can be separated on the basis of morphometric differences nor in males, neither in females (Appendix 3).

During the course of this project, while reviewing all

Table 1. Results of the principal component analysis (PCA) of 10 morphometric variables from 33 populations of *Incilius occidentalis* in Mexico. a), males loadings and cumulative percent of variation; b), males eigenvalues; c), females loadings and cumulative percent of variation, and d), females eigenvalues

a)

Number	*Eigenvalue*	*Percent*	*Cum Percent*	*ChiSquare*	*DF*	*Prob>ChiSq*
1	83.456	91.383	91.388	4381.588	54	0
2	2.9202	3.19760	94.580	771.207	44	1.4431E-133
3	1.742	1.908	96.489	472.863	35	3.83521E-78
4	0.937	1.026	97.516	268.854	27	1.08137E-41
5	0.709	0.777	98.293	185.844	20	6.93995E-29
6	0.606	0.664	98.957	121.856	14	2.72202E-19
7	0.339	0.371	99.329	42.854	9	2.29191E-06
8	0.288	0.315	99.645	22.697	5	0.000385519
9	0.170	0.186	99.831	0.491	2	0.781996311
10	0.153	0.168	100	0	0	

b)

Variable	*Prin1*	*Prin2*	*Prin3*	*Prin4*	*Prin5*	*Prin6*	*Prin7*	*Prin8*	*Prin9*	*Prin10*
SVL	0.857	−0.510	−0.011	−0.000	−0.016	0.012	0.038	0.003	−0.042	−0.010
HL	0.215	0.321	0.400	−0.486	0.316	−0.563	−0.174	0.060	−0.029	−0.026
HW	0.271	0.483	0.350	−0.208	−0.211	0.605	0.176	−0.284	−0.039	0.029
TL	0.326	0.552	−0.734	0.073	−0.037	−0.137	−0.108	−0.053	0.045	−0.076
TYMP	0.031	0.142	−0.026	0.036	0.080	−0.026	0.660	0.556	−0.453	−0.131
EYELID	0.066	0.063	0.045	0.096	0.283	−0.001	0.439	0.106	0.832	0.058
PAROTOID	0.125	0.202	0.365	0.550	−0.589	−0.354	−0.057	0.140	0.104	−0.013
IOD	0.107	0.157	0.198	0.609	0.640	0.191	−0.245	−0.039	−0.177	−0.110
SND	0.046	0.068	−0.036	0.074	0.070	−0.030	0.014	0.072	−0.125	0.979
END	0.050	0.047	0.007	−0.153	−0.071	0.363	−0.481	0.752	0.192	−0.001

c)

Number	*Eigenvalue*	*Percent*	*Cum_Percent*	*ChiSquare*	*DF*	*Prob_ChiSq*
1	131.603	89.587	89.587	3250.287	54	0
2	5.449	3.709	93.296	753.641	44	5.8102E-130
3	4.146	2.822	96.119	548.266	35	1.84544E-93
4	2.062	1.403	97.523	305.024	27	7.24154E-49
5	1.051	0.715	98.239	183.179	20	2.31416E-28
6	0.873	0.594	98.834	147.899	14	1.89116E-24
7	0.712	0.485	99.319	114.217	9	2.03082E-20
8	0.613	0.417	99.736	79.204	5	1.23086E-15
9	0.243	0.165	99.902	9.8307	2	0.007332808
10	0.143	0.098	100	0	0	

d)

Variable	*Prin1*	*Prin2*	*Prin3*	*Prin4*	*Prin5*	*Prin6*	*Prin7*	*Prin8*	*Prin9*	*Prin10*
SVL	0.883	−0.449	−0.025	−0.099	−0.031	−0.016	−0.047	−0.045	−0.014	0.019
HL	0.195	0.478	0.131	−0.373	−0.723	0.128	0.132	0.120	0.009	−0.061
HW	0.271	0.617	−0.014	−0.333	0.553	−0.194	−0.112	−0.259	−0.095	−0.024
TL	0.271	0.359	0.199	0.822	−0.119	−0.215	−0.097	0.039	−0.029	−0.084
TYMP	0.037	−0.004	0.468	−0.089	0.272	0.346	−0.290	0.641	−0.141	−0.246
EYELID	0.055	0.061	0.361	0.129	0.103	0.681	0.101	−0.456	0.316	0.228
PAROTOID	0.115	0.203	−0.768	0.166	0.041	0.508	−0.147	0.205	0.023	−0.053
IOD	0.101	0.037	−0.021	0.068	0.241	0.030	0.896	0.212	0.043	−0.272
SND	0.045	0.078	0.020	0.040	0.052	0.027	0.186	0.264	−0.423	0.838
END	0.054	0.078	−0.020	−0.058	0.087	−0.234	−0.054	0.371	0.829	0.309

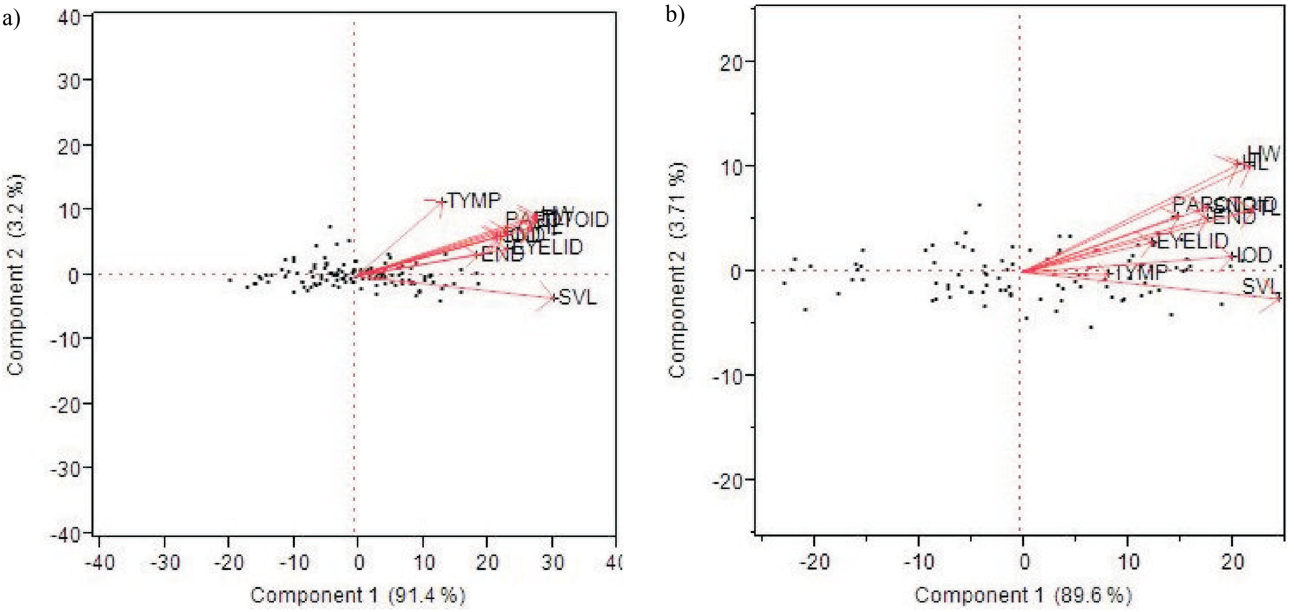

Figura 2. Two dimensions plot representation for 10 morphometric variables resulting from a Principal Component Analysis (PCA) performed in 33 populations of *Incilius occidentalis* in México. Direction and size of arrows indicate the value of each variable to explain the whole variance; A) males plot, B) females plot.

populations referred to *I. occidentalis,* the population from Chihuahua was identified as a new species (Santos-Barrera and Flores-Villela, 2011). Two other populations were identified as different and will be considered later (Santos-Barrera and Flores-Villela, in prep.). The remaining populations share the same morphological external design; all these populations are recognized as *Incilius occidentalis*, the redescription is presented below.

Incilius occidentalis Camerano, 1879

Bufo intermedius Günther, Catalogue of the Batrachia Salientia in the Collection of the British Museum. pp. 1859.

Bufo occidentalis Camerano, Atti. Accad. Sci. Torino, 14: 886-889, 1879.

Bufo intermedius Dugès, La Naturaleza, 1: 137-145. 1869.

Bufo monksiae Cope, Proc. Amer. Philos. Soc., 18: 263, 1879.

Bufo simus Schmidt, Denkschr. Akad. Wiss. Wien. math-nat., 14: 254-255, 1857; Kellogg, Bull. U. S. Nat. Mus. 160: 1-224, 1932. Smith and Taylor, 1948. U.S. Natl. Mus. Bull (194):42-43.

Cranopsis occidentalis Frost, Grant, Faivovich, Bain, Haas, Haddad, de Sá, Channing, Wilkinson, Donnellan, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green, and Wheeler, 2006, Bull. Am. Mus. Nat. Hist., 297: 364.

Ollotis occidentalis, Frost, Grant, and Mendelson, 2006, Copeia, 2006: 558. By implication

Incilius occidentalis, Frost, Mendelson, and Pramuk, 2009. Copeia, 2: 418; Frost, 2008, Amphibian Species of the World Online, vers. 5.4: Required change due to seniority of *Incilius* over *Ollotis*.

Lectotype: MZUT An 210-2. An adult male from México collected by Federico Craveri. Locality and date of collection unknown.

Paralectotypes: MZUT An 210-1 and MZUT An 210-3. Both males from Mexico, collected by Federico Craveri. Collection locality and date unknown too. See below type locality.

Diagnosis. *Incilius occidentalis* can be recognized by the following combination of characters: *1)*, medium to large-sized individuals (SVL range, males: 52.37-84.15; females: 76.42-91.93); *2)*, snout truncate in profile; *3)*, adult individuals with weakly developed cranial crests; *4)*, supraocular crest most developed, postocular crest evident in large individuals, parietal crest only evident in the largest individuals; *5)*, parotoid gland elliptical, medium sized (about 51% of HL), separated from eyelid by short series of granules; *6)*, tympanum conspicuous, round; *7)*, vocal slits in males unilateral or bilateral; *8)*, dorsal surfaces of adults covered with numerous medium-sized sharply pointed granules with keratinized tips, dorsal surface of juveniles smooth; *9)*, lateral descending line of tubercles absent; *10)*, dorsal coloration consisting of olive brown background with distinct pale cream vertebral stripe defining 2 dark brown lateral blotches, vertebral

Table 2. Analysis of variance (ANOVA) for the scores resulting from the principal components analysis (PCA) of 10 morphometric variables of males (A) and females (B) from 33 populations of *Incilius occidentalis* in Mexico. Variables significantly different at 95% confidence are marked with an asterisk (*). See text for details

a)

PC number	*DF*	*Sum of squares*	*Mean square*	*F Ratio*	*p> F*
1	17	2008.328	118.137	1.819	0.033*
2	17	67.941	3.996	1.349	0.1748
3	17	84.229	4.954	4.235	0.0001*
4	17	54.177	3.186	5.056	0.0001*
5	17	17.039	1.002	1.465	0.1192
6	17	15.214	0.894	1.736	0.045*
7	17	8.689	0.510	1.678	0.0563
8	17	7.432	0.437	1.675	0.056
9	17	2.370	0.139	0.895	0.615
10	17	6.529	0.384	2.900	0.0004*

b)

PC number	*DF*	*Sum of squares*	*Mean square*	*F Ratio*	*p> F*
1	16	3769.526	235.595	2.330	0.0067 *
2	16	118.630	7.414	1.695	0.0632
3	16	90.343	5.646	1.151	0.3238
4	16	107.288	6.705	4.882	0.0001*
5	16	32.609	2.038	1.908	0.0305
6	16	11.461	0.716	0.898	0.5735
7	16	18.400	1.150	1.753	0.0521
8	16	14.687	0.917	1.631	0.0782
9	16	2.3410	0.146	0.603	0.8725
10	16	3.279	0.204	1.466	0.1320

line interrupted at mid body in many individuals, and *11)*, ventral surfaces cream or pale yellow with no marks except in young individuals where pale gray dots may be present (Fig. 3).

Comparisons with sympatric and other related Bufonid species. Incilius occidentalis can be distinguished from sympatric species of the genus *Anaxyrus* by the dorsal color pattern, which consists of a medium light brown line often interrupted and bordered by lateral continuous olive to dark brown irregular stripes (versus numerous dark grey-green blotches on a light grey background in *Anaxyrus cognatus*, and numerous small brown dots on a light grey to brown background in *A. compactilis*). The body size of adults is noticeably larger in *A. cognatus*, males of *Incilius occidentalis* reaching 87 mm in SVL (versus 115 mm in *A. cognatus*). *I. occidentalis* can be distinguished from *A. compactilis* by of the soft texture of the dorsal skin which is covered with medium sized granules, smaller and more abundant at the flanks and limbs (uniformly rugose skin because of the presence of abundant small pointed granules). From *A. mexicanus*, because of the development of the cephalic crests, well developed supraoculars, postocular reduced and inconspicuous parietals in *I. occidentalis* (versus supraoculars less developed and parietal often lacking in *A. mexicanus*). Dorsal color pattern is reddish brown with scattered small dark brown dots sometimes suffused to form blotches in *A. mexicanus*. From *A. punctatus* differs because of the size and shape of the parotoid glands, elliptical and well developed in *I. occidentalis* (versus small and rounded). The color pattern of *A. punctatus* is highly variable, presenting a light grey to olive or reddish brown background with numerous red to brown dots almost always on a black ring at the base. Other *Incilius* species are not sympatric with *I. occidentalis*. In the phylogenies of North American *Incilius* and *Anaxyrus* species by Pauly et al (2004) and Bufonidae by Van Bocxlaer et al. (2010), *I. occidentalis* is the sister species of *I. alvarius*. *I. occidentalis* can be separated from this last species by differences of dorsal color pattern which consist of dull olive to gray ground without lateral blotches and scattered marks in *I. alvarius*, this pattern contrasts with the brown pattern of blotches of *I. occidentalis* described above. In addition, *I. alvarius* exhibits dorsal smooth skin with conspicuous enlarged glands on the thighs (Oliver-López, 2009), both features not present in *I. occidentalis*. Pyron and Wiens (2011) relate *I. occidentalis* as the sister taxon of *I. tacanensis*, this last species differs from *I. occidentalis* mainly because the

Figura 3. *Incilius occidentalis*, MZFC 6536, an adult male from 2 km East of Río Estórax, Peña Miller, Querétaro, Mexico, collected by Oscar Flores-Villela in October 12, 1993 (Photograph: O. Flores-Villela).

color pattern and granulation of dorsal surfaces, including dark brown bands bordered by lighter lines with a light mid-dorsal line in *I. tacanensis*, characters not present in *I. occidentalis*, moreover, a line of small conic granules runs along the flanks in *I. tacanensis*.

Description of the lectotype. An adult male with small body, SVL 56.92 mm. The head is truncate in lateral view and almost triangular in dorsal view; supraocular crest distinct, postocular and parietal crests less conspicuous; parotoid gland ovoid, length 55.62% of HL; tympanum distinct, nearly round, 30.67% of the diameter of the orbit; dorsal surfaces are covered with abundant large rounded granules with numerous pores, keratinized spines on the tip are severely damaged because of the storage and time of preservation; granules on the ventral surface uniformly distributed including the inguinal area; forelimbs short; fingers slender, webbing absent; internal palmar tubercle is rounded with dark brown pigmentation; external palmar tubercle is piriform, larger than internal palmar tubercle; relative length of fingers 3>1>4>2; nuptial excrescences on fingers 1-3, conspicuous, dark brown; subarticular tubercles distinct, paired on the first finger; supernumerary tubercles paired on the third and fourth digits; hind limbs are slender; length of toes in decreasing order is 4, 3, 5, 2, 1; webbing formulae, right foot **I**1-1**II**1-1**III**1 $^{1}/_{2}$-3**IV**3-1**V**, left foot **I** 1-1 **II** 1-2 **III** 2-3 **IV** 3-2 **V**. Short rows of keratinized tubercles on lateral surfaces of tarsi of feet; internal metatarsal tubercle large, 2X larger than the external metatarsal tubercle. Vocal slits bilateral.

Coloration in preservative. Background of the dorsum of body, including head and limbs, pale olive (olive gray, 42) with a distinctive pale mid dorsal stripe forming 2 paravertebral dark brown bands (olive brown, 28). There are 2 transverse parallel brown stripes on each side of the head. Lateral surfaces with several irregular small blotches. Parotoid glands are the same color as the rest of the dorsum with darker markings. Dorsal surfaces of hind limbs with transverse brown stripes. Ventral surfaces are cream and immaculate. The juvenile individuals have small black or dark gray markings on the throat and belly. Ventrally, fore limbs and thighs are immaculate.

Coloration in life. Adult specimens of *I. occidentalis* have a dorsal color pattern that varies gradually between individuals, with olive or unmarked brown being the most common background color (colors 42 and 44), and with a distinct pale mid-dorsal stripe forming two paravertebral dark olive to brown bands (colors 28 and 129); the vertebral stripe is often interrupted at mid body or below. Ventral surfaces are generally cream color (54) to light yellow with no marks.

Variation. Background color and lateral blotches on the dorsum can be darker in some individuals, especially the younger ones. The granulation of dorsal skin surfaces is clearly pronounced in juveniles, less abundant and sharply reduced in old individuals. Both paralectotypes resemble lectotype in dorsal and ventral color patterns. Webbing formulae of the hind feet can slightly vary, especially in relation to fingers 3, 4 and 5, but no differences exist between populations or through sexes. Variation in size of the body across sexes is remarkable, the largest average in SVL recorded in males is population 31, central Veracruz (82.32) and the lowest population 23, southeastern Oaxaca (53.84). Regarding females, the highest SVL recorded is population 16, Michoacán center (88.03), and the lowest from population 11, lowlands of Guerrero (66.85). During field work in Aguascalientes, a large female reaching SVL of 112 mm was collected; this individual was eliminated from the dataset to avoid biases in the statistical analyses.

Tadpoles. The tadpole has not been described.

Distribution, ecology and conservation. *I. occidentalis* occurs in northern and central Mexico from the highland Plateau in the states of Durango, Zacatecas, and Aguascalientes to the south and west through east Jalisco and Colima, to Central Mexico along Querétaro and northeastern Guanajuato, south and westward to Michoacán, Guerrero and central Puebla, to the east reaching central Veracruz and northern Oaxaca (Fig. 1). This species occurs in a great variety of environments, however it seems to prefer arid and semiarid scrub, mainly composed of mesquite (*Prosopis* spp.), huizache (*Acacia* spp.), and *Opuntia* (Zamudio et al., 1992). In some elevated areas above 1 000 m at the states of Durango, Guanajuato and Oaxaca it occupies pine-oak and oak forests (pers. obs.). It is possible to find individuals of this species in both pristine and moderately disturbed areas, often maintaining large populations in areas not too proximal to human settlements (Santos-Barrera and Urbina-Cardona, 2011). All observations suggest that this is a nocturnal species, avoiding high daytime temperatures by sheltering beneath rocks and logs or even under the ground. During the night, it is possible to find adult individuals adjacent to permanent or temporary springs and low rivers, commonly hidden beneath the shrubs and in the roots; it seems to avoid permanent ponds and pools. This is a stream dwelling species, in the rainy season (July to September) in northern Mexico it is possible to observe aggregations of toads along the rivers shores. In the arid and semi-arid areas of Puebla, adult individuals may be active until November and early December. Diet of adult individuals mainly consists of ants, orthopterans and several other insect larvae, according to observations of stomach contents (Santos-Barrera, unpublished data). This species is classified as Least Concern in the Red List of the IUCN (IUCN, 2012), and it does not appear in any other conservation listings. However, although it is considered as a widespread species, numerous populations along its range have experienced continuous declines, almost always due to habitat loss and persistent disturbance of native forested areas for logging, to open areas for cultivation and human settlements, this has been especially observed in central Mexico (Santos-Barrera, pers. obs).

Taxonomic comments. According to Taylor (1951), one important character to separate the species *Bufo simus*, from *Bufo monksiae* was the absence of the *ostia pharyngea* as well as the hidden tympanum. Smith (1952) also mentioned the presence of this character when relates *Bufo tacanensis* with *B. occidentalis* since both species examined by him have *pharyngeal ostia*. However *B. occidentalis* presents a hidden tympanum while *B. tacanensis* lacks this feature. Smith (1952) did not mention the locality of the *B. occidentalis* examined to compare with *B. tacanensis*. The *ostia pharyngea*, was not examined in this study, and regarding the presence and size of the tympanum, this feature seems to be an uninformative character because it might be or not evident in this species. The only populations that consistently lack tympanum are 10 and 11, the Guerrero highlands and Guerrero lowlands, respectively. It was also noted that males may have 1 or 2 vocal slits without a discernible pattern of distribution of this character. The type locality of this species was not specified by the collector F. Craveri or the author Camerano (1879), based on the comparisons of numerous individuals with the type specimens, that clearly coincide with central Mexico morphotypes, and based on the speculated route of collector Craveri (Gavetti, pers. comm), it is recommended to follow Smith and Taylor (1948) criterion to define the type locality to Guanajuato city and surroundings.

Discussion

The use of external morphological characters and morphometrics is a useful tool to describe and identify species (Mendelson et al., 2012). Even more, these features can be related with environmental data to search for evolutionary patterns and life history traits and to explore into phylogenetics (Rosso et al., 2004; Zelditch et al., 2000). In the present study, morphology and morphometrics have contributed to nearly complete the resolution of the long-standing taxonomic enigma of the *I. occidentalis* complex thus completing the Alpha taxonomy. The study of morphological features in *I. occidentalis* lead to the recognition of the population of Chihuahua as a different species, but the morphometric structure in this last species has almost the same body proportions of the rest of the *I. occidentalis* populations. In terms of morphometrics, it is evident that only small differences exist among populations of *I. occidentalis* along its geographic range. These differences are patent mainly in males, as have been noticed in other *Incilius* species (Mendelson, 1998; Mulcahy and Mendelson, 2000; Mendelson et al., 2005). In this study, the largest male individuals were identified in southern Mexico, (population 31, southern Veracruz) averaging 82.32 of SVL, and 88.03 (population 16, central Michoacán), hence, largest males do not correspond to largest female populations. Neither small size populations have coincidence (Fig. 1).

Morphology and morphometrics in bufonid toads can be useful to make interspecific comparisons, or to check for variation into highly variable intraspecific species (Mendelson et al., 2011). Revising the global results of this study it is possible to conclude that there is not an identifiable geographic morphometric pattern among the populations of this species studied, as it has been described in other lowland Bufonid species (Mulcahy et al., 2006). These results could be the consequence of the generalist habits of *I. occidentalis* that can occur in a great variety of environments, (see the natural history section), at a wide elevation range from 200 m to 2500 m, dwelling almost always on the ground, beneath rocks and logs and/or burrowing in holes in the ground. At the present time, none of the known populations can be separated only on the basis of morphometric variables, which is not surprising given the conservative body shape documented in many bufonid species (Maxson, 1984; Mendelson et al., 2011). In fact, the family Bufonidae is complex, with a superficial similarity. This conservative morphological pattern in concordance with some ecological traits could promote their diversification and expansion from the South American morphotype (VanBocxlaer et al., 2010; Zug, 1993).

The PCA was selected as the first approach to explore the morphometric data of *I. occidentalis* because this analysis constructs new variables that better resume the observed variation (McKillup, 2012), which was particularly helpful in this study. It is well known that body proportions in anurans reflect 4 basic locomotor categories (Pough et al., 1998). The PCA demonstrated that both, males and females of *I. occidentalis* retain the standard anuran body shape that fits well into the walker-hopper type, with short forelimbs and medium-long hindlimbs (Pough et al., 1998). By examining carefully plots of PC 1 vs. PC 2 for males and females (Fig. 2), it is evident that populations form just one group, no outliers or dispersed points can be seen, thus forming a narrow ellipse where points are concentrated. The PCA showed that the general measurement of body, expressed as SVL explain the majority of the variation observed, this could be the cause of this concentration of points, other variables as HW, HL, and TL are highly positively correlated with SVL, then, a large snout to vent length is associated to a long head or to a long tibia. This was expected to some extent, since allometric effects were controlled by excluding juveniles or immature individuals. Commonly, in a PCA the first component explain 70 to 75% of the variance, and the second and third the 15 and 5% respectively (McKillup, 2012). In this study, for males, the PC 1 explains 91.4 % of the variance and the second just 3.2%; this is an unusual situation that can be explained by the conservative morphological pattern of Bufonidae mentioned above, this determines an isometric type that is conspicuous in both, males and females. The comparisons with the Tukey HSD test showed that all populations can be grouped by morphometric differences in no more than 4 groups, thus confirming a stable morphometric design (Appendix 3). This result has been recognized in other anuran species where relative proportions of the body remain stable even when an increase in body size and sexual dimorphism is detected (Duellman and Trueb, 1990; Mendelson et al., 2005).

The clarification of *I. occidentalis* systematics is in progress, by the moment, it can be concluded that *I. occidentalis* is a widespread species. Variation in the morphology is not reflecting taxonomic variation in the group. It is impossible to define a clear tendency in color pattern of dorsal and ventral surfaces; this is the same situation for distribution of granulation and development of cranial crests. Morphological features examined are insufficient to define species limits, besides the ones already recognized. Low levels of variation persist in characters such as coloration of dorsal and ventral surfaces. No consistent variation exists in the development of cranial crests and parotoid shape and size. The taxonomic importance of the toes and fingers size and development of webbing is so reduced that their information was used only for descriptive purposes. The rest of the variables show similar patterns, just few individuals are variable, even in the same population; other differences between individuals can be attributed only to age and development with no clear geographic trend, as is commonly observed in other bufonid toads (Mendelson et al., 2011). This is also the case of the tympanum; this structure can be evident or non-evident in individuals of the same population regardless of sex. However, measurement of tympanum was evaluated in all populations, except of populations 10 and 11 from Guerrero where individuals of both sexes consistently lack this character. Other relevant data that should be studied in this species complex are those related to natural history, including tadpole descriptions, call variation and habits as well as biogeography and evolutionary history. Knowledge of the phylogenetic relationships and species limits in the *I. occidentalis* complex is still in progress (Santos-Barrera and Flores-Villela. in prep). Different molecular studies recovered *I. occidentalis* and *I. alvarius* as sister taxa, this last species is an inhabitant of the Sonoran desert and recently recorded in Chihuahua, northern Mexico, thus controversially relating two geographic and morphological dissimilar species. The complete picture of the phylogeny and biogeography in *I. occidentalis* can certainly clarify this and other evolutionary questions (Pauly et al., 2005; Mendelson et al., 2011; VanBocxlaer et al., 2010).

Acknowledgements

I want to thank Dr. Elena Gavetti and Dr. Franco Andreone from the Museo Regionali di Scienzi Naturali, University of Turin (MZUT), for the loan of the type specimens of *I. occidentalis*. Thanks also to the curators of the following Museums who kindly provided the scientific material: AMNH (American Museum of Natural History), BYU (Brigham Young University, Monte L. Beam Museum), EBUAP (Escuela de Biología, Universidad Autónoma de Puebla), MZFC (Museo de Zoología, Facultad de Ciencias, UNAM), IBH (Instituto de Biología-Herpetología), TCWC (Texas Cooperative Wildlife Collection), ENCB (Escuela Nacional de Ciencias Biológicas-IPN), (UTA), University of Texas at Arlington, (UIMNH), University of Illinois Museum of Natural History (INHS), Illinois Natural History Survey. For field assistance I want to thank Luis Canseco, Francisco

Vargas, Alberto Mendoza and Gustavo Fernández. M. J. Hernández, Juan Fornoni and Armando Martínez advised with the statistical analyses. Heliot Zarza and E. Saturno helped with preparation of figures and tables. Unlimited support in many ways was provided by Oscar Flores-Villela. Financial funding for this project was provided by Conabio Project L244 (GSB and OFV) and two fellowships to GSB, DGEP-UNAM and AMNH, New York.

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Appendix 1. Population acronyms and numbers (see figure 1 for geographic location):

1.AGS-SW, south-western Aguascalientes
2.AGS-ZAC, Aguascalientes-Zacatecas border (center)
3.COL, Colima
4.DGO-ZAC, Durango Zacatecas border (central)
5.DGO-CTR, Center Durango
6.DGOE, Eastern Durango
7. EDOMX, Estado de México center
8.EMXSW, south-western Estado de México
9.GTOCTR, center Guanajuato
10.GROH, highlands of center Guerrero
11.GROL, lowlands of Guerrero
12.HGO, Hidalgo
13.JALCE, east-central Jalisco
14.JALE, eastern Jalisco
15.JALNE, north-eastern Jalisco
16.MICH, Michoacan center
17.MICHNE, north-eastern Michoacán
18.MOR, Morelos
19.NAY, Nayarit
20.OAXCTR, center of Oaxaca
21.OAXN, northern Oaxaca
22.OAXS, southern Oaxaca
23.OAXSE, southeastern Oaxaca
24.PUEN, northern Puebla
25.PUES, southern Puebla
26.QRO, Queretaro
27.SINDGO, Sinaloa-Durango border
28.SLP, San Luis Potosí west-center
29.SONNE, north-east Sonora
30.TLX, Tlaxcala
31.VRCTR, central Veracruz
32.ZACCTR, north-central Zacatecas
33.ZACSW, south-western Zacatecas

Appendix 2. *Additional specimens examined.*

AGUASCALIENTES: MZFC 10101-10103, 10105 Calvillo río Gil KU 29771,29772, 18 mi W Aguascalientes; UAA 00044-1, 0044-2, 00046-1-00046-3, presa La Codorniz, Calvillo, 00058, 00102, Aguascalientes; CHIHUAHUA: BYU 14542, Cerocouhni; BYU 39373, río San Miguel; KU 44432, 37 km S and 2.4 km E Creel, Barranca del Cobre; KU 47233, 8 km S Riíto; KU 47234, 3.5 km S Riíto; KU 52058-61, 52064-75, 5 km NW Temoris; KU 52062-63, 13 km SW Cuiteco; KU 56168, Urique; KU 63659-62, 4 km N Urique; KU 63663, Yosachique; MZFC 9931,-9933, 16536, 19813, 19814; UMMZ 111513 (a series of 10 specimens), UMMZ 117778 (a series of 2 specimens) and UMMZ 117779 (series of 4 specimens), Maguarichic; UMMZ 111515 (series of 4 specimens), Mojarachic; UMMZ 111114, La Polvosa; MVZ 46639-40, río Gavilán, 11 km SW Pacheco. COLIMA: LNLJ R- 267 Minatitlán, UMMZ 79971, N de Quesería; LACM 37091, 5.2 mi SW Tonilá; UMMZ 79971, justo al norte de Quesería; DURANGO: CAS 121000, 20 mi E borderline Sinaloa, CAS 169726 36 mi W El Salto; ENCB 10767-10769, 1.6 km SW La Peña; IBH 2711, 2711-2, La Michilía, 2832 rancho La Peña; KU 38212-38216, río Melones, 44536, 44537, 10 mi SW El Salto, 78312, 19.2 km NE Santa Lucía, 182528, 182529, 23.2 km NW El Palmito, LACM 88059, Revolcaderos, 105630 14 mi W El Salto; MZFC, 10082-10086, 10088, 10091, 10093, 10094, río Melones; UMMZ 113678, Pueblo Nuevo, 123023, 1.8 mi NW El Palmito; ESTADO

DE MÉXICO: AMNH 166814, 4 mi SW Aculco, CB 9022, Santiago de Malinaltenango, KU 67819, Ixtapan de la Sal; MZFC 3758, Almoloya de Alquiciras, MZFC 12629, 12629, Parque Sierra de Nanchititla. GUANAJUATO: CAS 87172, 7 mi NE Guanajuato; IBH 5046, 2 km N León; MZFC 1746, 1747, Guanajuato; TCWC 40597, 20 mi W Xichu; USNM 26160, 26161. GUERRERO: MZFC 1380, Jalapa; MZFC 1383, barranca del Tío Chico Reyes, Mpio. Zumpango del Río; MZFC 1399, Aprox. 100 m de Jalapa; MZFC 3752, Ixcateopan de Cuauhtémoc, Km 26.5 carreteta Taxco-Ixcateopan; MZFC 3753, Ixcateopan de Cuauhtémoc, Las Peñas; MZFC 3755, Tetipac, Los llanos, Km 10 Carr. Taxco-Tetipac; MZFC 3756, Km 14.4 Carr. Taxco-Ixcateopan; MZFC 3757, Km 8 Carr. Taxco-Ixcateopan, Taxco; MZFC 3759, Tetipac, El Peral, Km 10.2 Carr. Taxco-Tetipac; MZFC 12626-12627, Atlixtac, MZFC, 3760, Ixcateopan de Cuauhtémoc; MZFC 5800, Km 21.1 carretera Taxco-Ixcateopan; MZFC12642-12650, 12 km S Atlixtac; IBH 952, Omiltemi; IBH, 4284, 5 Km E Chilpancingo; IBH 7101, 10 mi SW Chichihualco; TCWC 10940, 4 mi W Mazatlán, 8000 pies; TCWC 16560, 3 mi N Colotipla; TCWC 10024, 2 mi SW Omiltemi, 7900 pies; TCWC 182534, 73.1 Km NE Jilguero; KU 67820, 14 mi S Ixtapan, El Mirador, 5100 pies; KU 182530-32, 37.7 Km SW Filo de Caballo, 1945 m; KU 105519, entre Chilapa y km75, E Chilapa; KU 182533, 73.1 Km NE Jilguero, 2106 m; ENCB 6515, Hueycatenango; HIDALGO: AMNH 6657, Guerrero, 59241; IBH 310, near Actopan; ENCB 6923, 6924, Tasquillo, 8309, 8311, near Huichapan, 11475, 11476, 11479, 11480-11485, 11501, 11600-11602, Mezquititlán, 11614, Huejutla, 12046, Nicolás, 12050, Huisticola, 12640, Taxquillo; MVZ 109501, Parque Nacional el Chico, MZFC 5826, 2 km E Aserradero, UMMZ, 56482, Velasco, 106398, 10 mi N Agua Blanca; USNM 148903-148905, 6 mi S Ixmiquilpan; JALISCO: ENCB 14781 Atemayac, 14782, Brizuela, 14784 Juanac; IBH 5940, 5941-5941-6, San Fernando Opio. Mascota, 6080-1-28, Villa Hidalgo; KU 27253, 27256, 2 mi N Guadalajara, 38217, 1 mi S Telostitlán, 67821 17 mi ESE Tequila, 67824, 26 mi WNW Magdalena, 73805 15 km W de Ameca, 73806 32 km NW Ayutla, 73807, 3 km NE Talpa. MICHOACÁN: MZFC 1519 y 1519-3, Zitácuaro, Coatepec de Morelos, San Francisco; MZFC, 19811-19812; IBH 4504, 23 Km E Morelia; UMMZ 94278-85, Tancítaro, 2 mi E Apo; UMMZ 112773-774, 2 mi S Jaranilla 5000 pies; UMMZ 112775, Cerro de Barolosa 8900 pies; UMMZ 104398, 2 mi E Coalcomán, sierra de Camachines, 4300 pies; UMMZ 104399, 0.5 mi NE Coalcomán, cerca del río Coalcomán; UMMZ 104796, 2 mi S Coalcomán; UMMZ 104400, 3 mi W Coalcomán; UMMZ 112776 (serie de dos ejemplares), cerro de Barolosa, 9000 pies; UAA 0004, Mpio. Morelia, río Chiquito; ENCB 2378, 20 Km E Morelia; ENCB 2804, El Paraíso de Purúa, Jungapeo; ENCB 13034, 9.5 Km S, 2 Km E Epitacio Huerta; ENCB 18 Km N, 12 Km E Coalcomán; ENCB 15373, 15378, 15382 y 15384,7 Km N, 10 Km E Araró; OAXACA: MZFC 1452 y 1453, Zapotitlán, alrededores; MZFC 3264 y 3264-2, 4 Km E Chapulco; MZFC 6080,6 Km E Tamazulapa; MZFC 12073-12077, 12 km NW Tlaxiaco; IBH 359, Huajuapan de León; IBH 7104, Camotlán; IBH 7105, 4.3 mi S Tonaltepec; IBH 7377, presa de Praxedes, 2.5 Km E Praxedes; AMNH 71125, Mitla; AMNH 72636, 11 mi SW Sola de Vega, 7000 pies; AMNH 72637, arroyo San Felipe, enfrente de Oaxaca; TCWC 55655, 4.3 mi S Tonaltepec, 6800 pies; KU 137526, 6.9 Km NE Oaxaca, 1500 m; KU 137527, 13.1 Km N Juchatengo, 1010 m; KU 137528, río Jalatengo, 8 Km S Jalatengo, 1280 m; MVZ 138943, 18.5 Km S Sola de Vega; ENCB 10339, Teumaxtla. PUEBLA: AMNH 13896-7, Santa Catarina, 64661 río Atoyac, cerca de Puebla; ENCB 11583, 21.3 Km S, 5.2 Km W Tehuacán; ENCB 13620, 3 Km NW San Martín Texmelucan; IBH 356-358, Tehuacán; 2775, San Juan Raya; KU 65543, 39808,7 mi S, 58239-58250, l4.4 Km W Huachinango; KU 97645, 97646, 1.6 Km N Tehuacán; KU 97647, 2.1 Km N Tehuacán; 97649, 97650, 97651, 3.2 Km N Tehuacán; 97652, 4 Km N Tehuacán; 97653, 4.5 Km N Tehuacán; 97654, 4.8 Km N Tehuacán; 97655, 11.2 Km SE Tepeaca; MZFC 2367, 2367-2, 10057-10059 Zapotitlán Salinas; UAP 114-10, Amozoc, 10 Km E Puebla; UAP 253-92, UAP 40-900, Zapotitlán de las Salinas; USNM; 115538-540, USNM 116544, Tecamachalco; QUERÉTARO: MZFC 1454, 0.5 Km NE Peña Miller; MZFC 3110, río Estórax, 15 Km SW Pinal de Amoles; MZFC 5864, Mesa de León; MZFC 6536-6538, San Juanito, 2 Km E Peña Miller, en el río Estórax; MZFC 6539-6555, Mpio. Cadereyta, Rancho Nuevo, río San Juan, en los límites con el estado de Hidalgo; IBH 4056, Ahuacatlán, Mpio. Pinal de Amoles; IBH 7112, Maconi; IBH 7113 Los Piñones; TCWC 33010, 17 mi E Cadereyta; TCWC 33022, 0.5 mi NE Peña Blanca; TCWC 33023, 10 mi E Cadereyta; TCWC 38377, 1 mi N Peña Blanca; TCWC 38433 y 38434, 1 mi N Peña Blanca; TCWC 40581 y 40585, 1-2 mi ENE San Pablo; ENCB 10668, Maconi. VERACRUZ: AMNH 13761, 13762; KU 23851; ZACATECAS: IBH 5063, 20 km N Valparaíso; MZFC, 7200, 5 Km S Tabasco, Carretera Zacatecas-Guadalajara, ENCB 14109, Cañitas, 14729 2 km SW San Juan Capistrano; KU 102690 17.6 km NW Jalpa; MZFC 5023, 13 Km W de Moyahua, 10107, Juchipila, 10111-10120, Valparaíso; UMMZ 118751 8 mi S Moyahua; USNM 47170 sierra Madre.

Appendix 3. Results from the Tukey-HSD test for components scores resulting from a PCA of 10 morphometric variables resulting significantly different from the ANOVA test of 33 populations of *Incilius occidentalis*. The component is designed above the table. a) Males comparison. b) Females comparison. Populations without letters assigned should be considered as different.

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a)

PCA 1

Population	*Column A*	*Mean*
14	A	13.455
2	A	6.975
15	A	4.514
16	A	3.475
13	A	2.993
30	A	2.891
26	A	2.193
20	A	1.944
25	A	1.303
32	A	−0.732
24	A	−0.827
12	A	−1.216
22	A	−2.402
28	A	−5.520
1	A	−5.568
27	A	−6.643
6	A	−11.868
33	A	−13.793

PCA 3

Population	*A*	*B*	*C*	*Mean*
33	A	B	C	2.437
30	A	B		1.357
15	A			1.296
2	A	B		1.285
14	A	B	C	0.694
24	A	B	C	0.492
13	A	B	C	0.445
20	A	B	C	0.439
1	A	B	C	−0.018
25		B	C	−0.046
32	A	B	C	−0.094
22	A	B	C	−0.343
26			C	−0.536
27	A	B	C	−0.666
6	A	B	C	−0.838
12			C	−0.891
28		B	C	−1.205
16	A	B	C	−1.642

PCA 4

Population	*A*	*B*	*C*	*D*	*E*	*Mean*
24	A					1.299
13	A	B	C			0.724
25	A	B				0.599
30	A	B	C	D		0.297
27	A	B	C	D	E	0.125
28	A	B	C	D	E	0.066
16	A	B	C	D	E	0.026
20		B	C	D		0.009
22	A	B	C	D	E	−0.004
26			C	D	E	−0.261
1		B	C	D	E	−0.363
6	A	B	C	D	E	−0.373
12			C	D	E	−0.442
2		B	C	D	E	−0.502
15				D	E	−0.660
32	A	B	C	D	E	−0.677
14	A	B	C	D	E	−0.849
33					E	−2.957

PCA 6

Population	*Column A*	*Column B*	*Mean*
14	A	B	0.502
30	A		0.355
12	A		0.314
15	A		0.144
26	A		0.073
20	A	B	0.023
2	A	B	−0.019
28	A	B	−0.023
25	A	B	−0.058
1	A	B	−0.116
16	A	B	−0.181
24	A	B	−0.291
27	A	B	−0.462
13	A	B	−0.494
6	A	B	−0.570
32	A	B	−0.584
22	A	B	−0.620
33		B	−2.604

PCA 10

Population	*Column A*	*Column B*	*Column C*	*Mean*
14	A	B	C	0.739
16	A	B	C	0.589
13	A			0.408
1	A	B	C	0.201
26	A	B		0.170
30	A	B	C	0.153
24	A	B	C	0.121
27	A	B	C	−0.002
25	A	B	C	−0.003
12	A	B	C	−0.024
22	A	B	C	−0.098
32	A	B	C	−0.126
28	A	B	C	−0.155
6	A	B	C	−0.178
2		B	C	−0.265
20			C	−0.277
15			C	−0.283
33	A	B	C	−0.316

b)

PC 1

Population	*A*	*B*	*Mean*
15	A		10.390
28	A	B	9.717
2	A		9.129
26	A	B	6.400
1	A	B	6.107
6	A	B	4.782
31	A	B	4.180
20	A	B	3.987
4	A	B	1.424
32	A	B	−0.191
9	A	B	−0.790
13	A	B	−1.092
12	A	B	−1.198
25	A	B	−1.841
27	A	B	−5.930
19		B	−13.219
14	A	B	−16.814

PCA 4

Population	*A*	*B*	*C*	*D*	*Mean*
27	A				1.431
19	A	B	C		0.900
25	A				0.734
9	A	B	C	D	0.706
20	A				0.704
26	A	B			0.674
1	A	B	C	D	0.648
12	A	B			0.619
6	A	B	C	D	0.375
13	A	B	C	D	0.295
31	A	B	C	D	0.010
4	A	B	C	D	−0.319
28	A	B	C	D	−0.962
14	A	B	C	D	−1.264
32		B	C	D	−1.553
15			C	D	−1.744
2				D	−1.821

PCA 5

Population	*Column A*	*Mean*
4	A	1.300
26	A	0.790
25	A	0.573
19	A	0.382
9	A	0.352
32	A	0.352
13	A	0.252
28	A	0.221
12	A	0.178
15	A	−0.106
14	A	−0.243
6	A	−0.456
20	A	−0.464
2	A	−0.619
1	A	−0.672
27	A	−0.785
31	A	−1.041