

**CONTRIBUTIONS TO THE MORPHOLOGY OF
PLECTROHYLA MYKTER (ANURA: HYLIDAE),
WITH COMMENTS ON SOME CONTROVERSIAL
CHARACTERS AND THE DIAGNOSIS OF THIS SPECIES**

**Contribuciones a la morfología de *Plectrohyla mykter* Adler
(Anura: Hylidae), con comentarios sobre algunos caracteres
controversiales y la diagnosis de esta especie**

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ABSTRACT

We describe the tadpole, juveniles, and two adult specimens of *Plectrohyla mykter*. The tadpole of this species is unique among *Plectrohyla* by having labial tooth row formula 4(4)/6. We found that tarsal fold, thoracic fold, and rostral keel are present in *P. mykter* but not evident in adults, and that the skin thickness is intermediate in comparison to other species of *Plectrohyla*. We conclude that these characters are not useful for the diagnosis of *P. mykter* and propose a new diagnosis for this species.

Key words. Tadpole, Skin thickness, Hylini, Mexico.

RESUMEN

Describimos el renacuajo, el juvenil, y dos especímenes adultos de *Plectrohyla mykter*. El renacuajo de esta especie es único en *Plectrohyla* por tener formula dental 4(4)/6. Encontramos que el pliegue tarsal, el pliegue torácico, y la quilla rostral están presentes en *P. mykter* pero no son evidentes en adultos, y que el grosor de la piel es intermedio en comparacion al de otras especies de *Plectrohyla*. Concluimos que los caracteres pliegue tarsal, pliegue torácico, y quilla rostral no son útiles para diagnosticar a *P. mykter* y proponemos una diagnosis nueva para esta especie.

Palabras clave. Renacuajo, grosor de la piel, Hylini, México.

INTRODUCTION

The genus *Plectrohyla* was redefined by Faivovich *et al.* (2005) to include, among others, all species of the formerly recognized *Hyla bistincta* group plus few species of the

Hyla miotympanum group (*sensu* Duellman 2001). All these species are collectively recognized as the *Plectrohyla bistincta* group. Currently, there are 24 species in the *P. bistincta* group, all distributed in Mexico.

One of the lesser-known frogs of the *Plectrohyla bistincta* group is *Plectrohyla mykter* Adler & Dennis, 1972. This species is known from eight individuals and its tadpole is still undescribed. *Plectrohyla mykter* inhabits cloud forest habitat of the Sierra Madre del Sur west of Chilpancingo, Guerrero, at elevations between 1985 and 2520 meters.

Adler & Dennis (1972) described *Plectrohyla mykter* based on three individuals (i.e., one male, one female, and one juvenile). Mendelson & Toal (1996), after reexamining the adult type specimens, concluded that four characters of the diagnostic combination (i.e., rostral keel, skin thickness, thoracic fold, tarsal fold) were erroneous. Presently, it is unclear what is the correct condition of these characters in *P. mykter*, or whether these characters can be used to diagnose *P. mykter* from other species of *P. bistincta* group.

We recently obtained tadpoles, adults, and a developmental series of frogs of the *P. bistincta* group from several localities west of Chilpancingo, Guerrero. Based on the most recent diagnosis, they appear to be *Plectrohyla mykter*. Herein, we describe the tadpole, juvenile, and two adults of *P. mykter* and based on our observations, discuss what are the conditions of the characters rostral keel, skin thickness, thoracic fold, and tarsal fold in this species. We also evaluate whether a diagnosis of this species that includes these characters is still useful based on the newly obtained material, and what factors might explain the discrepancies between various authors concerning these characters.

MATERIALS AND METHODS

Two adult males and several tadpoles were collected from various localities in and near Carrizal de Bravo, Omiltemi, and Sierra de Alquitran, Guerrero, Mexico (see Examined Specimens). The adults were collected in

June 2002 and the tadpoles were collected in February and March 2004. Most tadpoles were reared through metamorphosis, except for a few that were preserved in formalin (10%) at various developmental stages, and one that was preserved in 95% ethanol. Liver samples were obtained from adults and juveniles, placed in 95% ethanol, and stored frozen.

For morphological comparisons, we examined the adult paratype of *Plectrohyla mykter* (KU 137552) and photographs of the head of the holotype (KU 137553). To assess the relative thickness of the skin of *P. mykter*, we measured and compared the skins (i.e., dermis plus epidermis) of one individual of this species (UMMZ 238295) to that of *P. bistincta* (UMMZ 112839[WED 7105]) and *P. chryses* (CAS 142943), two species that represent the extremes of skin thickness in this group. We obtained skin samples from the area above the suprascapula and sectioned them histologically (5 μ) using the paraffin method of Wessner (1960). The sections were stained with Hematoxylin-Eosin. Skin thickness was measured as the average of three independent measurements using a compound microscope with a calibrated ocular micrometer.

Tadpoles were staged following Gosner (1960). Terminology of tadpole morphology follows that of Altig & McDiarmid (1999). Measurements and terminology of adult morphology follow that of Duellman (2001). We define "fold" as a bend of the skin over on itself, so that one part of it covers another. The fold is described as "flexible" if it can be moved easily, and "flap-like" if it is itself folded.

To assess if the specimens examined comprise a single species, we used morphological and molecular comparisons. To assess DNA similarities between specimens from different populations and developmental stages, we sequenced and compared a part of the cytochrome *b* gene (361 base pairs) of

an adult (MZFC 18350) and a tadpole from near Carrizal de Bravo (UMMZ 238299) with that of juveniles from Omiltemi (UMMZ 238297) and Sierra de Alquitran (UMMZ 239828). We used the primers MVZ15 and H15149 to amplify the cytochrome *b* gene (Faivovich *et al.*, 2005) using standard PCR conditions. After obtaining DNA sequences, we used Genbank's BLAST algorithm to find the degree of similarity between these DNA sequences and others that are publically available. Museum acronyms: MZFC = Museo de Zoología Facultad de Ciencias, Universidad Nacional Autónoma de México; UTA = Amphibian collection of the University of Texas at Arlington; UMMZ = University of Michigan Museum of Zoology.

RESULTS

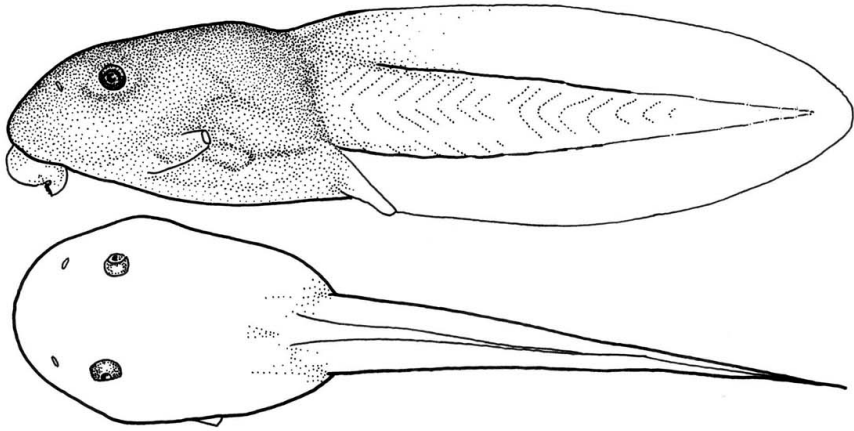
Table 1 shows the uncorrected pairwise DNA distances between an adult from Carrizal de Bravo (MZFC 18350) to specimens examined from different populations as well as several other hylid species. The new DNA sequences are deposited under Genbank accession numbers KR011919-KR011922 (Table1).

Description of the Tadpole of *Plectrohyla mykter*: A tadpole in stage 25 (UTA A-56511) (units in mm) (Fig.1): total length 31.6; body length 12.8; tail length 18.8; height of caudal muscle (at base of tail) 3.9; width of caudal muscle (at base of tail) 3.0; tail maximum height 9.1; maximum height of dorsal fin 2.9; maximum height of ventral fin 2.9; body width 7.4; body height 6.8; eye diameter 1.2; interorbital distance 4.7; nostril length 0.5; snout-spiracle distance 7.2; nostril-eye distance 2.0; oral disc diameter (transverse) 4.1.

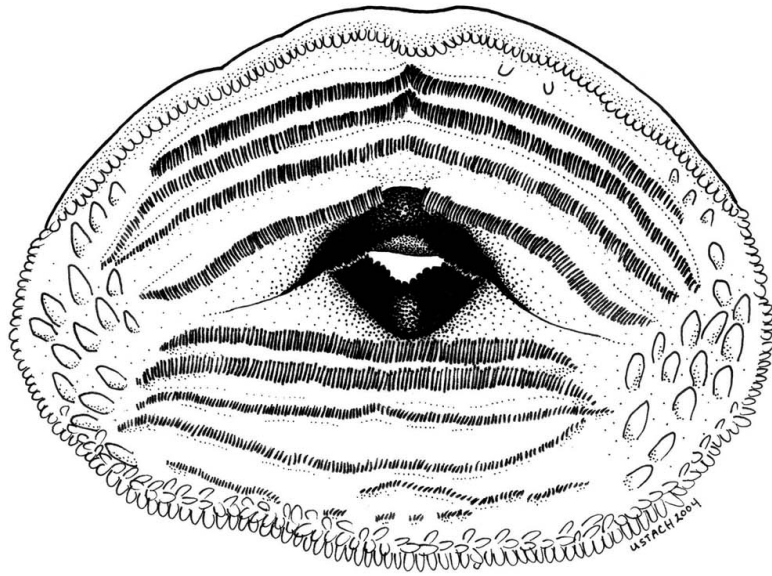
Body ovoid in dorsal view, widest at about level of eyes, wider than high, depressed in lateral view, higher posteriorly than anteriorly; neuromasts visible; snout rounded in dorsal and lateral views; eyes moderate in size, round, situated dorsolaterally; nostrils slit-shaped, directed anteriorly, closer to eye than tip of snout; spiracle sinistral, forming round aperture; vent tube dextral; caudal musculature robust, highest at base, gradually tapering to pointed tip; caudal fin tip rounded; dorsal and ventral fins of nearly equal height throughout their lengths.

Table 1. DNA distances of different populations of *P. mykter* and other Hylidae to *Plectrohyla mykter* (MZFC 18350).

Species	Sequence ID	stage	Population	DNA distance
<i>Plectrohyla mykter</i>	MZFC 18350 (KR011922)	Adult	Carrizal de Bravo	-
	UMMZ 238299 (KR011919)	Tadpole	Carrizal de Bravo	0.6%
	UMMZ 239828 (KR011921)	Juvenile	Sierra de Alquitran	0.8%
	UMMZ 238297 (KR011920)	Juvenile	Omiltemi	3.0%
<i>P. calthula</i>	AY843841	Adult		10.0%
<i>P. bistrincta</i>	AY843834	Adult		10.2%
<i>P. arborescandens</i>	AY 843823	Adult		11.6%
<i>P. cyclada</i>	AY 843851	Adult		12.0%
<i>Smilisca cyanosticta</i>	AY 844008	Adult		14.4%
<i>Hyla japonica</i>	AB303949	Adult		15.5%



5 mm



2 mm

Figure 1. Tadpole of *Plectrohyla mykter* (Stage 25) (UTA A-56511 [JRM 4614]): (A) Lateral view and (B) Oral disc.

Oral disc large, not emarginate, completely bordered by single row of marginal papillae; submarginal papillae large, present at lateral tips of anterior and posterior tooth rows, becoming less numerous anteriorly and posteriorly, few extending along anterior margin of tooth row A1 (sinistral) or A2 (dextral); two rows of small submarginal papillae present along posterior margin of oral disc, not extending anteriorly beyond level of tooth row P4; labial tooth row formula 4(4)/6, A1 shortest, A2–A4 of approximately equal length; A4 gap narrow; P1–P6 slightly shorter than anterior tooth rows, P1–P4 approximately equal in length, longer than P5–P6; P5–P6 intermittent; labial teeth in rows P4P6 shorter in height than those in other rows. Upper jaw wide, jaw sheath serrate; lower jaw of medium width, sharply V-shaped; small keratinous flange present posterior to serrate margin of upper jaw sheath, parallel to jaw sheath, not serrate.

In life, body black, tail transparent. In preservative, dorsal and lateral surfaces of body uniform dull brown; ventral surface of body transparent, gut visible; caudal musculature and fins unpigmented.

A series of tadpoles in Stage 25 (UTA A-5650911) vary in body size (10.3–15.6 mm). The larger tadpoles tend to have greater development of the lateral submarginal papillae, with more papillae extending into the area anterior to the anterior tooth rows. Labial tooth row formula is 5(5)/6 or 5(5)/7. A-1, P5, P6, and P7 are variable in size and degree of completeness; P5 and P6 may be complete and well developed, may be markedly reduced in size (especially P6), or may be highly fragmented (intermittent). A1 and P7 are always intermittent and poorly developed. One individual in Stage 34 has well-defined dark blotches on tail musculature and fins. A tadpole from Omiltemi (UMMZ 238301) has a short, depressed body, labial tooth row formula 5(5)/6, small labial teeth in

all rows, large serrations on upper jaw sheath, and short anal tube.

Tadpoles were collected at night in a swiftly running, deep (ca. 1m), cold stream in virgin humid oak forests mostly in parts of the stream covered by overhanging vegetation, and during the day in a slow-moving stream between large boulders. The tadpoles collected during the day swam rapidly toward the boulders when disturbed.

Description of adults and juveniles of

Plectrohyla mykter: Two adult males (MZFC 18350, UMMZ 238295) (units in mm): SVL 39.4, 38.2; head length 11.1, 11.6; head width 13.2, 12.8; interorbital distance 4.8, 3.62; eye diameter 4.6, 4.3; tympanum diameter 1.3, 1.1; eye-nostril distance 3.1, 2.6; eye-tip of snout distance 5.7, 5.7; internarial distance 3.3, 3.5; eye-tympanum distance 1.5, 1.8; disc width of Finger III 1.8, 2.3; disc width of Finger I 1.1, 1.3; thigh length 17.8, 19.5; shank length 20.5, 20.1; foot length 17.3, 18.3; inner metatarsal tubercle diameter 2.0, 1.5; outer metatarsal tubercle diameter 1.0, 0.6; snout long, round in dorsal view and profile, sloping ventrally in profile, extending well beyond jaw in ventral view; canthus round, barely distinct; rostral keel absent (Fig. 2); eyes protruding, large (eye diameter 37–41.4% of head length); tympanum small (tympanum diameter 26–28.3% of eye diameter), concealed dorsally by supratympanic fold and posteriorly by skin; tympanic ring distinct, slightly elevated anteriorly, not elevated posteriorly; thoracic fold absent; four or five irregular, shallow thoracic grooves present; thoracic grooves interrupted medially; arms covered with tubercles dorsally and ventrally; ventral tubercles forming low ridge along external edge of ulna; tarsal fold not evident; outer metatarsal tubercle present; webbing formula on foot $II-2^{+}III^{+}-3^{+}III^{1/2}-3IV3^{-}-1V$ (MZFC 18350) or $II-2^{+}III^{+}-2^{1/2}III^{1/2}-2^{1/2}IV2^{1/2}-1^{+}V$ (UMMZ 238295); dorsum of body, head,

and limbs dark brown or pale olive-brown; green cast on flank and posterior surfaces of thighs; yellow spots on dorsum of fingers and toes; throat covered with peppering of black pigment; iris coppery brown.

Two juveniles (MZFC 19686; UMMZ 238297) have a small, wide, round rostral keel; rostral keel evident in dorsal view, not evident in ventral or frontal views; 3 to 4 thoracic folds present; anterior thoracic fold

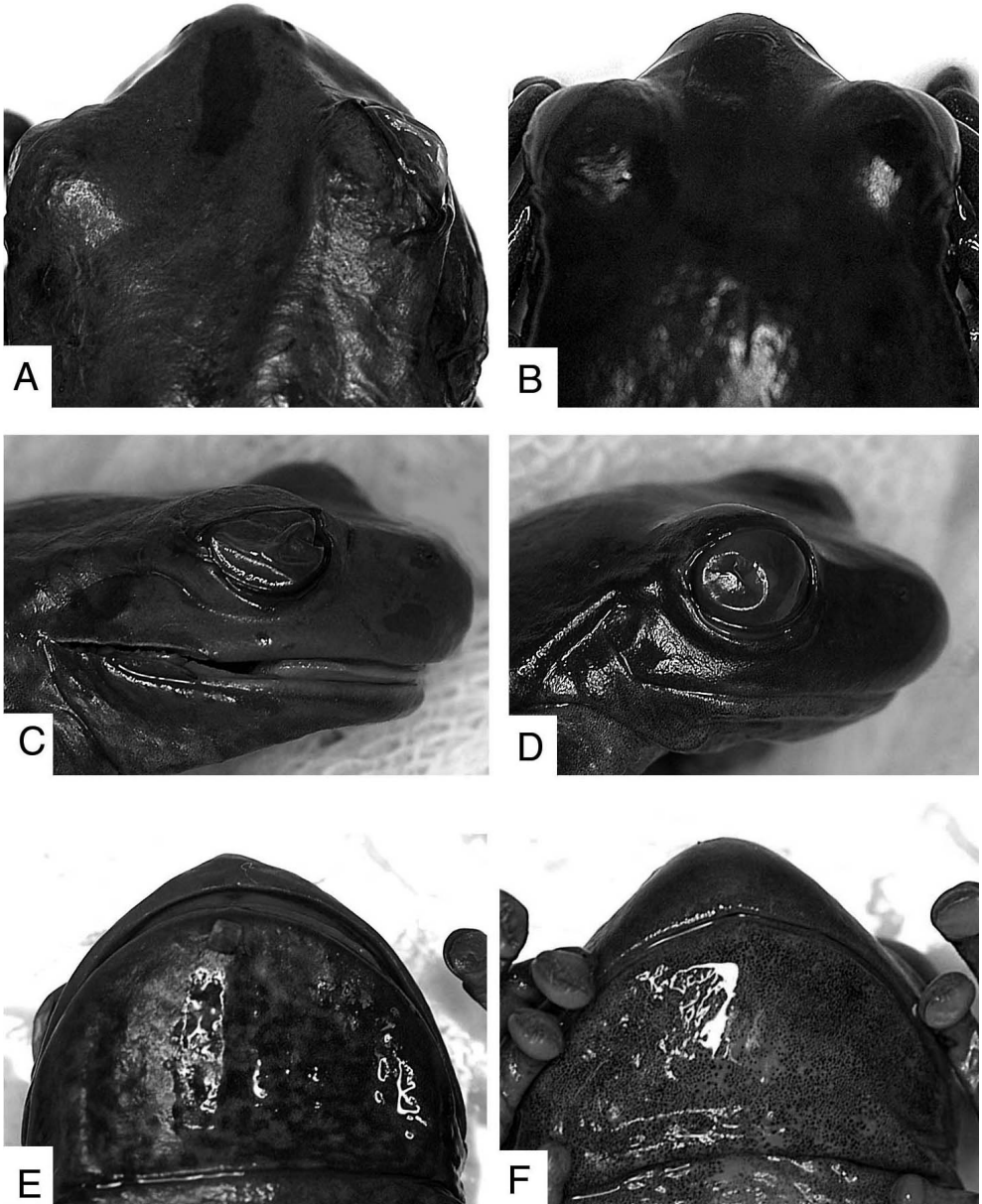


Figure 2. Dorsal, lateral, and ventral views of two adult individuals of *Plectrohyla mykter*. (A) (C), (E) female paratype (KU 137552); (B), (D), (F) adult male (MZFC 18350).

flap-like, medially interrupted, longer than the others; posterior thoracic folds flexible, not flap-like; dorsum of body, head, and legs smooth; dorsum and ventrum of arms tuberculate; ulnar ridge distinct, elevated, formed by large unpigmented or white tubercles; tarsal fold evident, elevated, with discrete, unpigmented tubercles; belly and ventromedial part of thighs shagreened; thorax and throat covered with tubercles; dorsum of body, head, and limbs beige or brown covered in various degrees and densities by green pigment interspersed with dark brown spots or reticulations; canthal, labial, and supraocular bands coppery or dark brown; flanks reddish brown dorsally, creamy white ventrally, with dark brown reticulations; posterior surfaces of thighs and arms dark brown with small reddish cream spots; chest and posterior part of throat cream with a brassy purple cast; belly, ventromedial area of thighs, and ventrum of arms purple; anterior part of throat brassy purple; throat covered with peppering black pigment; distal part of thighs, tibia, and foot dull yellow; iris golden or coppery. Older juveniles (UMMZ 23928-30) have dorsum of body, head, and limbs dark reddish brown with discrete black spots or mottled beige and black with discrete black spots; supratympanic, canthal, and labial stripes dark brown; flanks light brown with dark brown reticulations and small yellow spots; posterior surfaces of limbs dark brown with small yellow spots; tips of hands and feet brown or orange, with small, yellow spots on dorsum; belly dull purplish; ventromedial part of thighs pinkish, other parts yellow; throat and ventrum of arms dull yellow; throat covered with peppering black pigment; iris coppery.

DISCUSSION

Morphological similarities between tadpoles, metamorphs, juveniles, and adults from all the populations examined, plus the evidence from the developmental series, suggest that all the individuals collected are one species.

Additionally, Table 1 shows that all of the DNA sequences of the examined specimens are similar to one another (0-3% sequence divergence) but very distant from other available *Plectrohyla* sequences (at least 10% sequence divergence). There is close to 100% sequence similarity between the DNA of a tadpole and an adult from Carrizal de Bravo.

The DNA sequence distance between the specimens from Omiltemi and Carrizal de Bravo is rather large for species (3%) (Table 1) and their tadpole morphology is slightly different. These observations suggest that the populations from Omiltemi and Carrizal de Bravo might be different species or at least divergent populations. However, given that the DNA distance between two likely closely related species of *Plectrohyla* (e.g., *P. arborescandens* Taylor and *P. cyclada* Campbell & Duellman) is 8%, the morphological variation in tadpoles of *P. mykter* (especially from Omiltemi where $n = 1$) is not well understood, and all juvenile specimens are very similar, the current evidence leans toward classifying them as a single species until further information is available.

We assign all the collected specimens to *Plectrohyla mykter* because of a variety of shared phenotypic features, including that the adult males (MZFC 18350; UMMZ 238295) have SVL 38.2–39.4mm; skin thickness intermediate between thin and thick; snout rounded in dorsal and lateral views; rostral keel absent; tympanum evident; vocal slits absent; axillary membrane not evident; webbing on fingers vestigial; nuptial excrescences present on all fingers; ulnar tubercles forming ridge; webbing formula on foot $\text{II}-2^+\text{III}^+-3\text{III}^{1/2}-3\text{IV}3-\text{IV}$ (MZFC 18350); anal sheath grooved medially, opening at midlevel of thighs; thoracic and tarsal folds absent; posterior part of belly, chest, ventromedial part of thighs, and anterior part of throat deep purplish with

brassy pigment; flank and posterior surfaces of thighs black with yellow spots and green cast (Mendelson and Toal 1996; see Adler and Dennis 1972 for coloration characters).

The recently collected adult males (MZFC 18350; UMMZ 238295) differ from the type specimens of *Plectrohyla mykter* (conditions in parentheses) by having the snout protruding in profile and ventral views (not protruding); eye diameter 39% of head length (33%); tympanum diameter 11% of head length (19%); throat covered by peppering black pigment (mottled) (Fig. 2). These characters do not vary within the type or the assigned specimens, raising the possibility that the new specimens are not *P. mykter* but are in fact a new species; a better understanding of the intraspecific variation in *P. mykter* is needed to test this hypothesis.

Mendelson & Toal (1996) concurred with the original diagnosis of *Plectrohyla mykter* (Adler & Dennis 1972), except that they did not find a thoracic fold, tarsal fold, or rostral keel in adults, and found the skin of the dorsum of body to be thin, rather than intermediate between thin and thick.

We found that the skin thickness in *Plectrohyla mykter* (0.57 mm) is intermediate between that of *P. bistincta* Cope 1877 (0.73 mm), a thick-skinned species, and that of *P. chryses* Adler 1964 (0.12 mm), a thin-skinned species (Duellman, 2001). These observations validate Adler & Dennis's (1972) suggestion that the skin of *P. mykter* is only "slightly thickened" compared with species such as *P. bistincta*. These observations are also consistent with Toal & Mendelson's (1995) suggestion that within the *P. bistincta* group the thickness of the skin (i.e., on the dorsum) varies continuously between thick (in *P. bistincta*) and thin (in *P. chryses*). However, the character "skin thickness" is presently not useful for the diagnosis of *P. mykter* because the skins of most species of *Plectrohyla* have

never been measured (see Toal & Mendelson 1995, for additional comments on the validity of this character).

We found that the adult individuals of *Plectrohyla mykter* lack thoracic and tarsal folds, and that a rostral keel is present only in the female paratype (Fig. 1). We also found that all juveniles have an elevated tarsal fold, a flap-like thoracic fold, and a well-defined rostral keel. These findings confirm that thoracic fold, tarsal fold, and rostral keel are present in *P. mykter* and suggest that they disappear, or become less evident, during ontogeny. Thus, the characters "presence or absence of thoracic fold", "tarsal fold", and "rostral keel" are not currently useful for the diagnosis of *P. mykter* because our understanding of their taxonomic distribution within the *P. bistincta* group is poor; in particular, we do not know if any of the species reported to lack tarsal fold, thoracic fold, and rostral keel in adults have these characters as juveniles. Our observations in *P. mykter* show that this is a possibility.

On the other hand, the character "absence of tarsal fold in adults" is useful for the diagnosis of *Plectrohyla mykter*. This character has been reported in *P. cyanomma* Caldwell 1974, *P. celata* Toal & Mendelson 1995, *P. sabrina* Caldwell 1974, and *P. calvicollina* Toal 1994 (Duellman 2001). In contrast, the character "absence of thoracic fold in adults" does not appear to be useful for the diagnosis of *P. mykter* because we do not know if other authors have used the same narrow definition of thoracic fold that we used (see Materials and Methods); that is, we do not know if the species of the *P. bistincta* group considered to have a thoracic fold have instead a thoracic groove. Finally, the character "presence or absence of rostral keel in adults" is not useful for the diagnosis of *P. mykter* because we still do not know if the presence of a rostral keel in females and its absence in males is due to sexual, ontogenetic, or intraspecific variation;

we need to examine more material to resolve this issue.

With the removal of the characters of the thoracic fold, tarsal fold, rostral keel, and skin thickness (the exception is “absence of tarsal fold in adults”) from the diagnosis of *P. mykter* (Mendelson & Toal 1996), it is unclear if this diagnosis is still useful. Part of the difficulty in assessing whether the diagnosis of *P. mykter* is useful is the questionable nature of the same characters for the diagnoses of all the other species of the *P. bistincta* group (i.e., they are part of all the current diagnoses). However, it appears that *P. mykter* can still be distinguished from the other species of the *P. bistincta* group by the following combination of characters: SVL < 40mm.; snout rounded in dorsal and lateral views; tympanum evident; vocal slits absent; axillary membrane not evident; webbing on fingers vestigial; nuptial excrescences present on all fingers; ulnar tubercles forming ridge; webbing formula on foot II-2+III+3-III^{1/2}-3IV3-1V (MZFC 18350); anal sheath grooved medially, opening at midlevel of thighs; tarsal fold absent in adults; posterior part of belly, chest, ventromedial part of thighs, and anterior part of throat deep purplish with brassy pigment; flank and posterior surfaces of thighs black with yellow spots and green cast; tadpole with LTRF 4(4)/6. In light of the challenge to the characters rostral keel, skin thickness, tarsal fold, and thoracic fold, a reevaluation of the diagnoses of all the species of the *P. bistincta* group is in order.

Adler & Dennis’s (1972) conclusions about the characters “thoracic fold”, “tarsal fold”, and “rostral keel” in *Plectrohyla mykter* conflict with Mendelson & Toal’s (1996) conclusions (see also Duellman 2001), even though they examined the same specimens. For example, Adler and Dennis (1972) reported that the holotype of *P. mykter* (KU 137553) has a tarsal fold, thoracic fold, and rostral keel while Mendelson & Toal (1996)

reported these characters to be absent. Mendelson & Toal (1996) hypothesized that the discrepancies were due to “preservation techniques” (i.e., presumably the characters changed, due to the way the specimens were preserved, between the time of the original description of this species and the time they reexamine the types). However, this study shows that freshly collected adult males lack a rostral keel, tarsal fold, and thoracic fold suggesting that preservation artifacts may not be a plausible explanation for the discrepant conclusions.

The discrepant results among authors concerning the characters thoracic fold, tarsal fold, and rostral keel in *Plectrohyla mykter* resulted from different interpretations of the same observation. For example, the small protrusion between the nostrils of the paratype (Fig. 1) could be considered a rostral keel (Adler & Dennis 1972; this study) or not (Mendelson & Toal, 1996) and the thoracic grooves could be considered a thoracic fold (Adler and Dennis 1972) or not (Mendelson & Toal 1996; this study). The solution is to define the characters “tarsal fold”, “thoracic fold” and “rostral keel” more narrowly.

The tadpole of *P. mykter* differs from that of other species of the *P. bistincta* group with known tadpoles (i.e., all except *P. calvicollina*, *P. charadricola* Duellman 1964, *P. chryses*, *P. labedactyla* Mendelson & Toal 1996, *P. mihuatlanensis* Meik *et al.* 2006, *P. pachyderma* Taylor 1942, *P. psarosema* Campbell & Duellman 2000, and *P. sabrina*) by having LTRF 4(4)/6. Therefore, in the context of the phylogenetic hypotheses of Hyline by Faivovich *et al.* (2005) and Wiens (2005), LTRF 4(4)/6 appears to have evolved independently in *P. mykter* and the genus *Ptychohyla*.

Finally, Mendelson & Toal (1996) suggested that *Plectrohyla cembra* Caldwell 1974 may be a junior synonym to *P. mykter*. We disagree

because the male of *P. cembra* lacks nuptial excrescences in all fingers and its tadpole has LTRF 2(2)/3 (Caldwell 1974; Duellman 2001).

Comparative materials: *Plectrohyla mykter*: Mexico: Guerrero: near the Carrizal de Bravo-Chichihualco road, 4 kms W from the deviation to Asoleadero (MZFC 18350, UMMZ 238295, 238300); Asoleadero, 2520 m. (KU 137552); 3.7 km from Carrizal de Bravo via Chichihualco (UMMZ 238298); stream behind town of Carrizal de Bravo (MZFC 19685–6, UMMZ 238296, 238299); road Chichihualco–Puerto del Gallo at a point approximately 3.9 km E of Carrizal de Bravo (UTA A-56509–11, MZFC-JRM 4615, 4632, 4635); Omiltemi, in a stream near water tank (UMMZ 238297, 238301); Sierra de Alquitran (UMMZ 239828-30, 239961).

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