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# A new species of *Chrysina* Kirby (Coleoptera: Scarabaeidae: Rutelini) from Mexico based on morphological and molecular data

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**Abstract**
A new species of *Chrysina* is described from Guanajuato state, Mexico, including morphological and molecular characters based in genetic difference of cytochrome oxidase (*cox1*) mitochondrial marker. This new species belongs to a new called "Peruviana complex", which presents taxonomic problems due to the high morphological similarity and difficult allocation of the species *C. peruviana*. However the population of the Sierra de Santa Rosa de Lima, Guanajuato state, Mexico, has distinctive characters that separate it from all other populations.

**Keywords:** Peruviana complex, *cox1*, DNA barcoding, *C. peruviana*.

## 1. Introduction
The genus *Chrysina* Kirby is distributed only in the Americas, from the southern part of the United States of America through Central and South America (Ecuador) [1]. A great part of Mexican territory is covered by mountains; many of them have been isolated by natural processes for a long time and have created characteristic ecosystems that have served as endemism centers for many species [2]. That may be the reason why Mexico is the country that holds the biggest species richness of this genus, closely to 60 species, from where many remains undescribed due to the scarcity of specimens or because many of them can be morphologically similar, even in specific characters like male genitalia. A morphological and molecular identification of this new species has been made, that was misidentified by Morón and Márquez [3] as *Chrysina peruviana* Kirby from Guanajuato, finding some morphological characters that help in the diagnosis and a genetic distance in DNA barcoding marker [4], giving more data for this division. DNA barcoding (usually a fragment of the *cox1* mitochondrial DNA gene) can be helpful to solve some of taxonomic problems [4, 5]. This molecular technique has shown to be successful tool in identifying different groups of beetles [6, 7, 8].

The new species belong to the Peruviana group (Sensu Hawks) [9] including 19 species distributed in México, United States of America (*C. beyeri* Skinner) and Guatemala (*C. giesberti* Monzón).

## 2. Materials and methods
### 2.1. Field sampling
A total of 28 specimens belonging to the ingroup were collected in two nights using a pair of light traps with a distance of 300 meters between them; one specimen was found alive inside a log. The locality belongs at the Sierra de Santa Rosa de Lima, Guanajuato state, Mexico. Five specimens assigned to *C. peruviana* were collected under the same light tramp conditions at Hidalgo and Puebla, Mexico for comparing with the specimens of Guanajuato. All specimens were preserved in absolute ethanol and kept at -20 °C.

### 2.2. DNA extraction
We used 15 specimens (ten of Guanajuato and five assignated to *C. peruviana*) for the molecular analyses. All the specimens collected were used to extract the fragment corresponding to the DNA barcode marker cytochrome oxidase subunit I mt DNA gene. The tissue used was obtained from abdomen. The DNA extraction was made using DNeasy (QIAGEN®) extraction kit for blood and tissue following the manufacturer's instructions.

**2.3. Amplifications and sequencing of the selected DNA**

Fragments were prepared in a total volume of 15 µl, with 10.40 µl of $ddH_2O$, 3.0 µl 5x PCR buffer, 0.24 µl of each primer, 0.12 µl Taq polymerase (Bioline Taq), 1-2 µl of DNA template. PCRs were carried out using the primers VF1d_t1 (5´-TGTAAAACGACGGCCAGTTCTCAACCAACCACAARG AYATYGG-3´) and VR1d_t1 (5´-CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRA ARAAYCA -3´) [10]. PCR cycling conditions were as follows: an initial denaturing step of 1 min at 94 °C, followed by 5 cycles of 94 °C for 30 s, 50 °C for 40 s and 72 °C for 1 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 40 s and 72 °C for 1 min, a final extension step at 72 °C for 5 min [11]. PCR products were sequenced at the Instituto de Biologia, UNAM in both directions, edited with MEGA version 6.0 [12] and aligned manually. All of the *cox1* sequences generated are deposited in GenBank (accession numbers KU569567-77).

**2.4. Species delineation analysis**

The delimitation of Peruviana complex based on the barcoding locus was through of 2% corrected pairwise distance criterion, using the Kimura 2-parameter distance model (K80, nst=2) [13] and visualized with a Neighbor Joining (NJ) tree. Sequence divergences were obtained with the K80 distance model and neighbor joining tree were generated using MEGA 6.0 [12]. This approach has proved to be a rapid, generally reliable tool for identification of species in most animal groups [4].

**2.5. Morphological examination**

We revised type, representative paratypes and *C. peruviana* samples collected under the stereomicroscope. The specimens were photographed to illustrated and compared morphological characters. The photos were obtained with a Leica Z6APO macroscope and at the Instituto de Biologia, UNAM and the magnification used are specified in the legends of the figures.

**3. Results**

**3.1. *Chrysina aurilisternum* new species**

(Figures 1, 2, 3, 4, 5, 6, 10, 11, 12, 16, 17, 18, 19, 20).

**3.2. Type material**

Holotype male and allotype female, 26 more paratypes with the same collecting data. Holotype, allotype and 17 paratypes deposited in the Colección Nacional de Insectos (CNIN), UNAM; 9 specimens deposited in the private collections of authors

**3.3. Description**

**3.3.1. Holotype male** (Fig. 1-2) (N=10) All measurements in mm. Length 26.0-34.0; width at elytral humeri 13.0-16.0; maximum width 12.0- 18.0.

Dorsum green (Fig. 1) to orange greenish, can also occur as a chromatic orange aberration (Fig. 3), ocular canthi of same color. Antennal segments reddish brown, only the dorsal scape shine green; elytra with internal and external margins with a little more orange reflections; commonly, without melanic dots disperse (Fig. 1, 3) like present in *Chrysina peruviana* Kirby; apical umbone with same color (Fig. 17); pygidium dull green with orange reflections and shine green margin (Fig. 17). Venter green with golden or coppery reflections (Fig. 11). Legs with shine green trochanter, femora green to pinkish brown depending of light incidence, tibiae pinkish iridescent (Fig. 16) and tarsi light blue to violet (Fig. 6). Head dorsal surface densely punctated with small superficial pits (Fig. 4); clypeus convex with green to reddish margins in dorsal view and pinkish to coppery reflections in frontal view (Fig. 4); intraocular distance of two times to antennal club length; mentum slightly convex in anterior part, anterior depression reaching the medial, profuse punctuation more deeper and wide in the anterior half, with light setation at laterals (Fig. 5). Pronotum at base 2.3 times wide as interocular distance; microsculpture similar to frons; lateral margins well delimited with an orange greenish coloration, except in the anterior part when they are slightly diffused and green. Scutellum with orange reflections and same punctuation than pronotum and frons. Elytra (Fig. 1) 1.3 times longer than broad, with distinctly punctuated with a more deepest and widest pits, with a five vertical rows of punctuations very slightly marked, discernible in the stereoscopic microscope, from the sagittal line to apex, and one more close to lateral end of elytra; interstria punctuation of the same type and shape. Pygidium (Fig. 17) with a normal convex form, profusely punctuated with a deep pits and one white setae, only the anterolateral marginal pits without setae. Venter with mesosternal protrusion black and short (Fig. 10), rounded apex reaching to two thirds of mesocoxae; prosternal, mesosternal and metasternal pleurites and sternites shine green with pinkish or coppery reflections, but never reddish like present in *C. peruviana*, very setosae. Legs protibia tridentated (Fig. 6), with black external margin more developed in the two first protibial processes which are more close to each other than the third; hind femora no conspicuously swollen (Fig. 12), hind tibiae with a conspicuous concavity in the apical third (Fig. 16), better seen in ventral view; all the articles with dorsal and ventral punctuation. Male genitalia (Fig. 18-19) similar to *C. peruviana* (Fig. 23-24) in size, shape and form, reason for be considered by some authors like this last species, parameres elongated (Fig. 18), subparalels with blunt apex; ventral plates (Fig. 19) long without striation, semi convex towards the sagittal line, lateral margins spiniform, length 11 and 3 wide.

**3.3.2. Allotype female:** Similar than male except as follows: length 31-34; width at elytral humeri 14.0-16.0; anterior tarsi more slender, elytra lateral margins prominent at middle, pygidial plate dull green. Genital plates (Fig. 20) of 3.5 length and 5 wide, with sparse pale setae more frequent in the distal region, posterior tips darkened. Apparently one from each seven specimens is female in the population.

**3.4. Etymology**

The specific name *aurilisternum* is a composed word that refers the golden iridescent ventral plates and the intermediate word “li” from the locality name, Santa Rosa de Lima.

**3.5. Diagnosis**

*Chrysina aurilisternum* is a new member of the Peruviana group (sensu Hawks 2001). This species is part of a “peruviana complex” in which can be included one more new species from Puebla state based in molecular distance of cox1

mitochondrial marker. Is very similar to the traditional description of *C. peruviana* but can be differentiated by some morfological characters present only in this last species: different punctuation of head and clypeus (Fig. 7); reddish and more compact mentum (Fig. 8); protibial processes thicker (Fig. 9); mesosternal protrusion (Fig. 13) completely black, without covering cuticle; coxae, trochanter and ventral plate (Fig. 14) with reddish iridescence coloration, propleura completely reddish; absence of swollen femora in leg III (Fig. 15); straighter concavity in metatibia (Fig. 21); pygidial plate (Fig. 22) dull green to greenish brown. The new species can be recognized by a ventral green golden coloration with pinkish reflections, thoracic pleurites shine green to golden green, the elytra commonly without melanic dots and the differences presented in femora and tibiae of leg III, the male genitalia is very variable but generally with longer concavity between parameres.

We obtained 11 COI sequences (678 base-pairs) from six specimens assigned to *Chrysina aurilisternum* and five to *Chrysina peruviana*. This species also possess a genetic difference with *C. peruviana* in cox1 marker of 2.06-2.22% based in K80, and an intraspecific difference of 0.0-0.15% (Fig. 25).

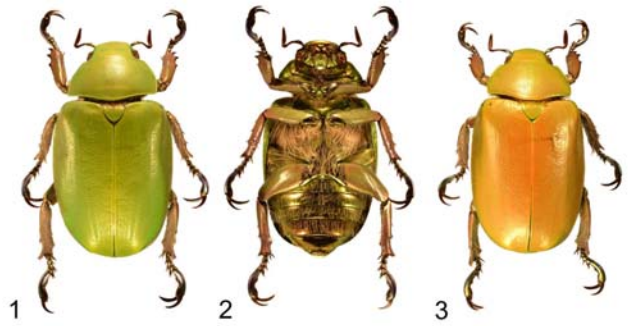



**Fig 1-3:** Dorsal and ventral habitus of adult *Chrysina aurilisternum*. 1-2) Male holotype with regular coloration. 3) Male paratype with orange form.

### 3.6. Distribution and Remarks

This species is restricted to Sierra de Santa Rosa, in Guanajuato state, Mexico. Is interesting that the vegetation is predominantly an oak-pine forest and this is the only species of the genus *Chrysina* living there.

## 4. Discussion

**4.1. Variation.** This species can show a chromatic variation from shine green, to dull green, including orange chromatic aberration in whole body. The male genitalia shows high intraspecific variation in the morphology of parameres, including the distance between them and deep of the central concavity, also the ventral plates can look different, especially in the sagittal part, being difficult to separate in some specimens with those of *C. peruviana*.

**4.2. Species delimitation.** Our preliminary results from mithochondrial marker support the morphology differences between those species analyzed. Also supported of the distributional data of *Chrysina aurilisternum*, which it has only been recorded at this location.

The cox1 sequences belonging to the "Peruviana complex" of three localities (with 2% corrected pairwise distance criterion) was obtained 2% or more of genetic difference, supporting the new species description from Guanajuato morphospecies from the rest (see Fig. 25). Also, this suggests the existence of at least two species for the group, but probably, they can be more. This hypothesis could be better supported including more specimens of the "peruviana complex" and additional molecular markers. The species delimited by the DNA sequence based approach it was congruent with *C. aurilisternum* morphospecies. These results therefore not only substantially increased the taxonomic knowledge of this group in the country, but also they are important for further working to solve taxonomical problems within the genus.

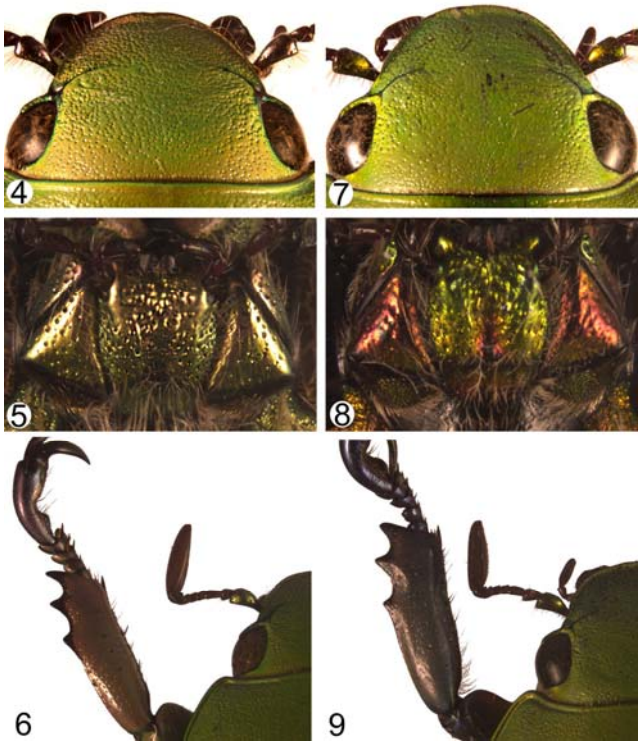
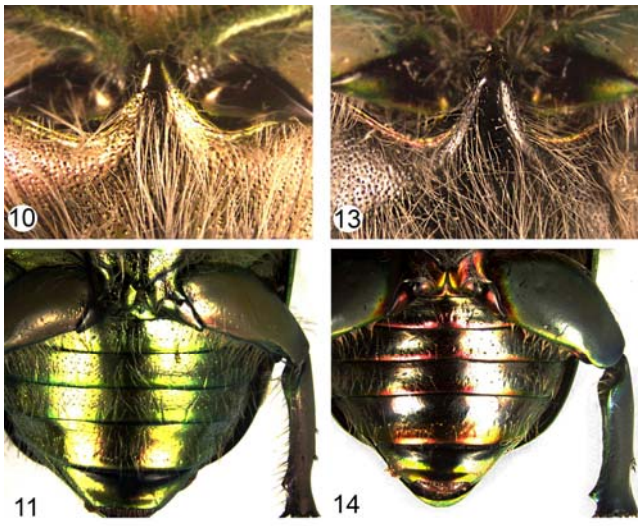



**Fig 4-9:** Male *Chrysina aurilisternum* (4-6) structures against male *Chrysina peruviana* (7-9). Clypeus 15.6x (4, 8). Mentum 20x (5, 8). Protibia 10x (6, 9).



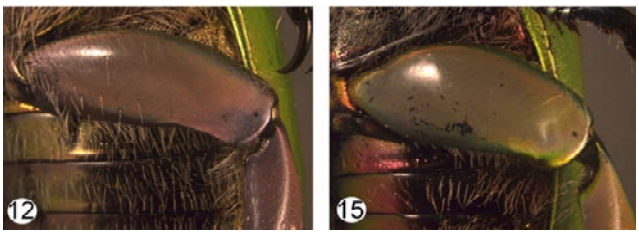
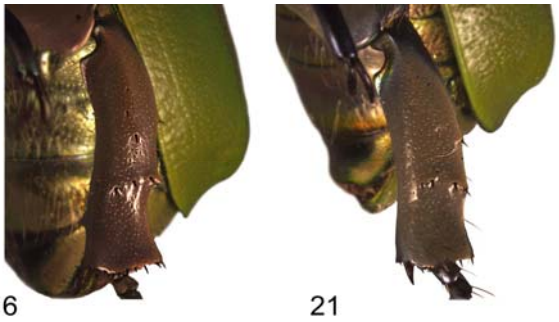



**Fig 10-15:** Ventral characters view. Male *C. aurilisternum* (10-12). Male *C. peruviana* (13-15). Mesosternal protrusion 12.5x (11, 14). Ventral plates 7.13x (11, 14). Hind femur 12.5x (12, 15).



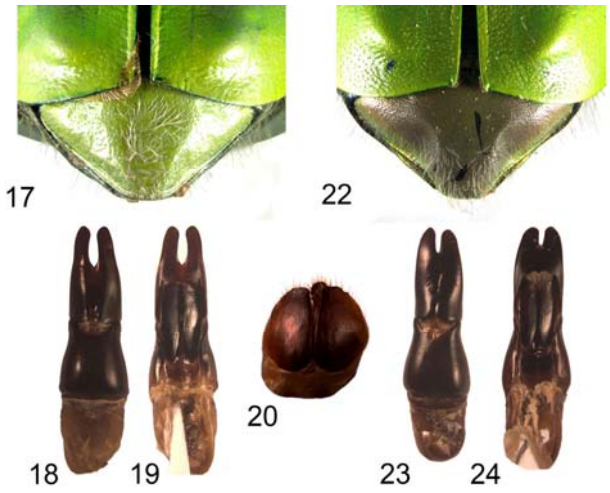




**Fig 16-24:** Genitalia and hind tibia comparison. Male *C. aurilisternum* (16-19). Female *C. aurilisternum* genital plate in dorsal view 10x (20). Male *C. peruviana* (21-24). Hind tibia 10x (16, 21). Pygidium 12.5x (17, 22). Genital capsule in dorsal view 10x (18, 23). Genital capsule in ventral view 10x (19, 24).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. C. aurilisternumC03 | | | | | | | | | | | |
| 2. C. aurilisternumC04 | 0.1559 | | | | | | | | | | |
| 3. C. aurilisternumC05 | 0.0000 | 0.1559 | | | | | | | | | |
| 4. C. aurilisternumC06 | 0.0000 | 0.1559 | 0.0000 | | | | | | | | |
| 5. C. aurilisternumC08 | 0.0000 | 0.1559 | 0.0000 | 0.0000 | | | | | | | |
| 6. C. aurilisternumC09 | 0.0000 | 0.1559 | 0.0000 | 0.0000 | 0.0000 | | | | | | |
| 7. C. peruvianaCp01 | 2.2207 | 2.0612 | 2.2207 | 2.2207 | 2.2207 | 2.2207 | | | | | |
| 8. C. peruvianaCp02 | 2.0589 | 1.8997 | 2.0589 | 2.0589 | 2.0589 | 2.0589 | 0.1560 | | | | |
| 9. C. peruvianaCp03 | 2.0589 | 1.8997 | 2.0589 | 2.0589 | 2.0589 | 2.0589 | 0.1560 | 0.0000 | | | |
| 10. C. peruvianaCp04 | 2.2207 | 2.0612 | 2.2207 | 2.2207 | 2.2207 | 2.2207 | 0.0000 | 0.1560 | 0.1560 | | |
| 11. C. peruvianaCp05 | 2.2207 | 2.0612 | 2.2207 | 2.2207 | 2.2207 | 2.2207 | 0.0000 | 0.1560 | 0.1560 | 0.0000 | |

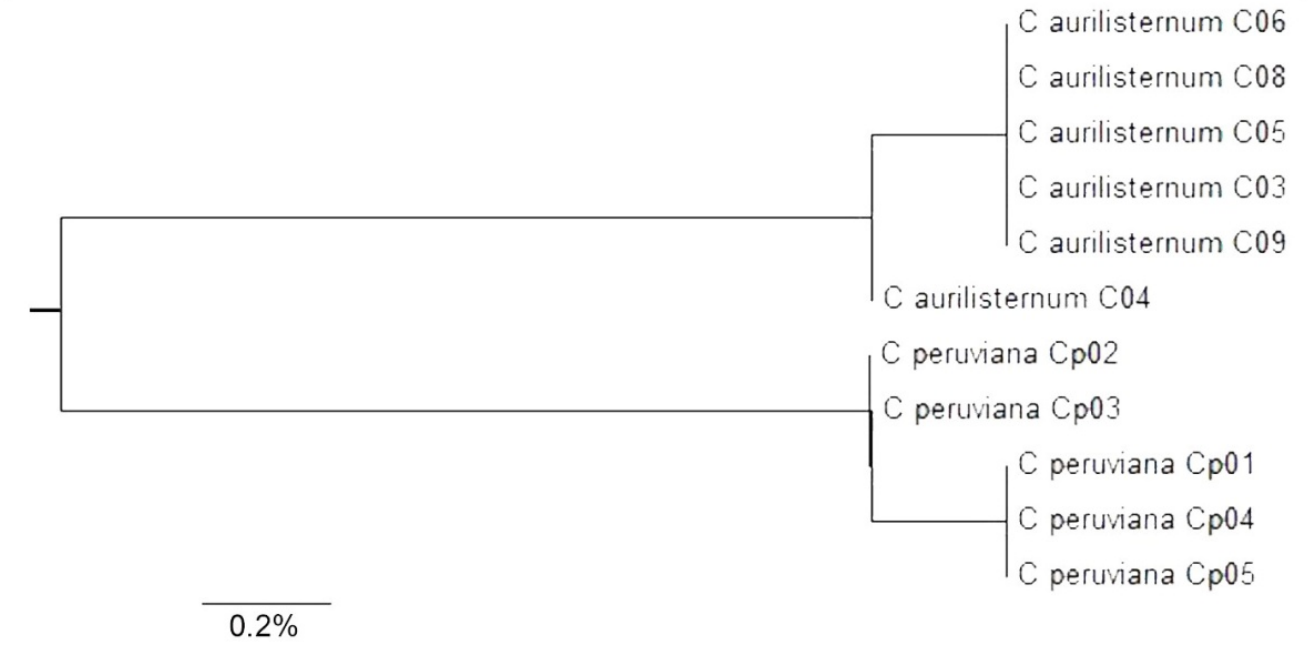



**Fig 25:** Genetic distances using the Kimura 2-parameter distance model and neighbor joining tree showing genetic distance between *Chrysina aurilisternum* and Chrysina *peruviana*.

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