

A new species of aposematic grasshopper of the Bolivian genus *Pseudoutanacris* (Caelifera: Gomphocerinae) ~~discovered from~~in the Andean cloud forest of the Ecuadorian Amazon basin

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Abstract

The lack of knowledge about the diversity of insects, especially in the tropics, is a significant gap that we aim to address in this study focusing on Orthoptera. Our research contributes to the identification of poorly understood species within the genus *Pseudoutanacris* and provides insights into related species. We have identified a new grasshopper species belonging to this genus in the montane forests of the eastern Andes in Ecuador. This discovery expands the known distribution of the genus, previously limited to a single species in the Bolivian tropics, by over 2,000 kilometers. The newly described species, *Pseudoutanacris grilla* **sp. nov.**, shares a unique coloration pattern with its congener, *Pseudoutanacris chromobapta* (Jago, 1971), setting them apart from other members of the Amblytropidini tribe. During our study, we also observed *P. grilla* **sp. nov.** on the same plant as *Megacheilacris graminicola* (Descamps & Amédégnato, 1971) (Bactrophorinae: Romaleidae), a species with similar chromatic characteristics. This finding also marks the first formal documentation of the new geographical records of *M. graminicola* in Ecuador.

Introduction

Despite being insects of relatively large size, striking colors, interesting shapes, and economic importance in agriculture, the Orthoptera group has been relatively understudied in the neotropics, and Ecuador is no exception. With a total of approximately 30,000 species known worldwide, of which 40% correspond to short-horned diurnal grasshoppers (Caelifera), the country's diversity is only about 2% of the world's total (Cigliano et al., 2025). In well-known groups such as vertebrates, plants, butterflies, or certain families of beetles, the percentage of diversity reaches values greater than 5% (Campos et al., 2020). This disparity highlights the

limited research efforts directed towards orthopterans, particularly in a diverse country like Ecuador, where vast areas such as the páramos-Páramos and montane forests of the Andes remain largely unexplored.

The Amblytropidini tribe, mainly distributed in the Neotropical region, is one of the 19 recognized tribes of the Gomphocerinae subfamily found almost worldwide, except in Oceania and certain circumpolar areas. Amblytropidiini is formed by nine genera and about 39 species (Cigliano et al., 2025). In Ecuador, the presence of the tribe is limited to just two species: *Fenestra platyceps* (Hebard, 1924^[gt1]); and *Peruvia nigromarginata* (Scudder, 1875^[gt2]); (Buzzetti & Carotti, 2008; Cigliano et al., 2025). This low diversity value likely indicates undersampling, suggesting that the country's biodiversity of this group may increase in the future.

The genus *Pseudoutanacris* (Jago, 1971) is currently composed of a single described species found in the tropical zone southeast of Bolivia, in the Department of Santa Cruz de la Sierra. Other photographic records extend its distribution to the southeast of Peru, in the Department of Madre de Dios (Bay, 2015). At first glance, *Pseudoutanacris* differs from all species of the tribe Amblytropidiini and even from the subfamily Gomphocerinae by its coloration, which is composed of bright tones and striking alternating colors, unlike the brown, cream, and orange tones that dominate in other species of the group (Jago, 1971).- The type species of the genus, *Pseudoutanacrisnautia chromobapta* (Jago, 1971), is known only from males, and its name reflects the colorful nature of a related genus^[gt3], *Utanacris* (Miller, 1934^[gt4]), of the subfamily Catantopinae from the Malay Peninsula.

At-In the end, this study marks the beginning of a series on the acridoid grasshoppers of Ecuador. The National Institute of Biodiversity of Ecuador has been investigating these grasshoppers for the past years, building on the research conducted by a French mission from the Museum of Natural History of Paris in the late 20th century.

Materials & Methods

All the specimens cited in this work are deposited in the scientific collection of invertebrates at the National Biodiversity Institute (INABIO) in Ecuador, representing the MECN (Ecuadorian Museum of Natural Sciences) collections. Labels of type material are quoted separately, line breaks are indicated by a backslash (/), and additional information is given between brackets ([]).

The specimens studied were collected under research permits No. MAAE-DBI-CM-2022-0228 and MAATE-DBI-CM-2023-0309 issued by the Ministry of Environment of Ecuador.

Specimens of the new species were compared with published descriptions of the species *P. chromobapta* (~~Jago, 1971~~), and photographs of the type specimen, available in the Orthoptera Species File (Cigliano et al., 2025). Measurements of the study material (holotype; and paratypes) were taken with a digital caliper (accuracy ± 0.1 mm). The total body length refers to the insect's body length, from the head to the tip of the abdomen. The width of the head was measured between the two outermost points of the head, ~~in the case of males the eyes; the eyes, in the case of males,~~ while in females, the measurement was taken from the posterior edge of the head.

The male genitalia were prepared following the procedure proposed by Hubbell (1932). The process begins by softening the entire animal in hot water for a short period (30 seconds) and focusing on the tip of the abdomen for a slightly longer time. Using a stereo microscope, an incision is made on the left side between the distal tergites and sternites.^[gt5] The membrane connecting the ventral surfaces of the paraprocts to the sclerotized plate at the cephalic end is then cut. The caudal end of the genital mass is carefully slid outward to expose the penis. The pallium is separated from the subgenital plate, and the entire genital apparatus is removed. A treatment with 10% KOH in a water bath^[gt6]for 2 minutes is performed ~~To~~ to study the genitals; ~~a treatment with 10% KOH in a water bath for 2 minutes is performed~~, followed by soaking the sample in distilled water. If necessary, soft tissues are cleaned, and the structures are preserved in 70% alcohol.^[gt7] A Zeiss Stemi 2000-C microscope and a Canon G10 camera were used for the genital study. Photographs of live animals were captured using a Nikon D3300 camera with a Nikkor 105mm macro lens and a Sony Alpha7 camera with a Sigma 105mm macro lens.

For molecular species identification, we amplified the classical animal DNA barcode, a fragment of the mitochondrial cytochrome oxidase I (COI) gene, in collaboration with the Canadian Centre for DNA Barcoding (CCDB). We used the C_LepFolF and C_LepFolR primers and ~~following~~ followed the standard protocols of the Biodiversity Institute of Ontario at Guelph University (Ratnasingham et al., 2024).^[gt8]

To genetically identify the male and female specimens of *Pseudoutanacris grilla* **sp. nov.**, we compared the acquired DNA barcodes of specimens (MECN-FC1987 (♂), MECN-FC1988 (♀), and MECN-FC1992 (♂)) using the BIN code. BOLD Systems assigns unique alphanumeric codes called "Barcode Index Numbers" (BINs) to define distinct genetic clusters in the entire BOLD Systems database (Ratnasingham & Hebert, 2013). NJ trees were generated in BOLD Systems based on Kimura two-parameter distances; and were viewed and edited using the software MEGA X and FigTree v1.4.4. For the outgroup, we use ~~to~~ *Peruvia nigromarginata* (~~(Seudder, 1875)~~ published in this paper) and *Amblytropidia mysteca* (Saussure, 1861^[gt9]) (Kumar et al., 2018; Ratnasingham & Hebert, 2013^[gt10]). Sequences are available in GenBank under the accession numbers PV173915, PV173916, PV173917, PV173918 in the BOLD Systems database (www.boldsystems.org/).^[gt11]

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: [urn:lsid:zoobank.org:act:1EE26CD7-7B6F-4894-8971-374CF07F9214]. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

Results

Pseudoutanacris grilla sp. nov.

<https://zoobank.org/NomenclaturalActs/1ee26cd7-7b6f-4894-8971-374cf07f9214>

Type material.-

Holotype: "♂" Ecuador. Morona Santiago, / M. El Tigrillo, road Macas- / Guamote 1920 m. / -2,217458, -78,224425 / 25-ago-2023 F. Campos²²; "[Depository:] MECN-FC-1687²²"; "[red label] HOLOTYPE / *Pseudoutanacris grilla*²²; "DNA voucher specimen / CCDB Lab code / Process ID / ORTEC164-24²²".

Paratypes: 4♂, 1♀ (nympha): Same data as holotype; "[Depository:] MECN-FC-1691-4, MECN-FC-1688²²"; "[yellow label] PARATYPE / *Pseudoutanacris grilla*²²; "data of DNA voucher specimen is same as holotype, ORTEC160-24, ORTEC168-24²²".

Etymology

The word "grilla" is derived from the Spanish word "grillo," which refers to the female of an orthoptera species known as "grillo" (Genus *Gryllus*) and taxonomically belongs to the infraorder Gryllidea. In Ecuador, most orthoptera are commonly referred to as "grillos" (crickets). The term is also used as an Ecuadorianism to describe a person who seeks attention, exhibiting in Ecuadorianism to describe a person who seeks attention and exhibits behavior similar to that of the species. [gt12]

Description.-

Male. Small to ~~medium-medium~~-sized insect with quite rough tegument.

Coloration: The insect has a predominantly green and red color ~~scheme~~ pattern (Fig. 1A). The head, pronotum disc, and wings are olive green, while the face also features yellowish tones (Figs. 1A; 2A-C). ~~Red~~ The red coloring is visible on the proximal half of the posterior femurs, as well as on the sides of the thorax and pronotum (Figs. 1A; 2A, C). The eyes appear blue in life (Fig. 1A). The antennae are black with a cream apex, light blue peduncle, and pedicel (Figs. 1A; 2A-C). The tibiae and tarsi of all legs are a faint turquoise color, with the femurs of the front and middle legs displaying a jade green hue with brown flecks. The distal half of the posterior femurs ~~show~~ ~~ease~~ ~~have~~ two faint green and cream bands near the black ~~knee~~ genicular lobes. The abdomen is orange on the sides and dorsally, with a yellowish lower part (Fig. 1A). The cerci are black, and the posterior tip of the abdomen ~~features~~ ~~is~~ light blue, white, and yellow tones (see Figs. 1A-3A-C).

Head: Slightly wider than long with prominent, almost oval eyes. The frontal costa ~~is very pronounced,~~ extending from the tip of the fastigium to below the middle ocellus, a distance similar to the width of the scapescape, and then ~~disappearing~~ disappears. Along its length, it is marked by small but deep subcircular points, arranged in two parallel rows in the holotype and more disordered in the paratypes (Fig. 2C-D). The ~~fastigium of the~~ vertex ~~of the fastigium~~ is truncated in front, with strong lateral and middle carinae, the latter extending moderately along the entire occiput (Fig. 2A-B). Antennae are less than 2.2 times the size-length of the head and pronotum combined, with 22 segments slightly narrower at their proximal end.

Thorax: The pronotum is rough on the dorsal and lateral surfaces, with three moderately marked sulci. The middle carina is evident throughout its entire length, while the lateral carinae are almost absent, except at the posterior end of the metazone. The posterior edge of the pronotum is angular and rounded, with a posterior projection of 25 degrees (Fig. 2A). The lateral ~~lobe~~ surfaces of the pronotum have a straight anterior edge, a slightly obtuse lower anterior angle, and a sinuous lower edge that is concave in the prozone and convex in the metazone. The posteroinferior angle is barely obtuse and appears subcircular. The posterior edge is barely sinuous, concave below, and convex at the upper end (Fig. 2C). The tegmina have very marked venation and are abbreviated with an extension that varies between the 8th tergite and the tip of the supra-anal plate. The hindwings are approximately the same size-length as the tegmina.

Legs: Long, with middle and forelegs almost as long as the hind femur extension, and the hind femur is $\frac{1}{4}$ longer than the tip of the abdomen. The hind leg has 10 internal and external tibial spines, with the inner ones approximately twice the size of the outer ones. The inferior inner lobe of the knee has an apical tip (Fig. 1A).

Abdomen: The abdomen ~~features~~ ~~has~~ ~~conical cerci that are robust and~~ robust conical cerci that project directly backward. The triangular epiproct has a slightly rounded posterior angle. In ~~top~~

dorsal view, the subanal plate is subtriangular with a rounded tip at the posterior end. In lateral view, it does not extend beyond the lateral edge (Fig. 3A-C).

Phallic complex: characterized by very long, straight, and narrow aedeagal valves. The epiphallus has a narrow and elongated bridge, spiniform anchorae with inward-directed tips, a triangular anterior process with a rounded outward-directed apex, and a dilated posterior process (Fig. 4A-D).

Female: The only female we have is an immature specimen, but we are including its description as it adds to the knowledge of the species. This is particularly significant as it is the first female description for this genus.

The female is twice the size of the male and has a wider overall appearance, especially in the head and thorax, giving it a fusiform shape. Compared to the male, the female has smaller antennae and posterior femurs. The antennae are 1.5 times the length of the head and pronotum, while the posterior femurs are 57% of the body size, whereas in males, this value is 88%. The female shares most anatomical features with the male, except for the presence of a prominent lateral carina on the entire pronotum. The most noticeable difference is the female's cryptic coloration, which is olive green with brown hues on the head, thorax, and extremities. The hind legs have black areas on the femur internally and externally, as well as on the knees-genicular lobes and the proximal part of the tibiae. The rest of the tibiae and tarsi are deep red. The antennae are mustard yellow with black spots towards the distal end and yellow in the last segments.

We obtained 3 complete COI sequences from three *Pseudoutanaeris-P. grilla* sp. nov. specimens from Ecuador. The barcode analysis results for specimens MECN-FC1987 (♂), MECN-FC1988 (♀), and MECN-FC1992 (♂) support their genetic similarity between the female and the males. Confirm these specimens are of the same species even though their colors and morphology are somewhat different. The Molecular clustering is available in supplementary file number 1.

Comparative diagnosis

Pseudoutanaeris-P. grilla sp. nov. is distinguished from *P. chromobapta* (Jago, 1971) (the only known species of the genus) primarily by its coloration. *P. grilla* sp. nov. has a green head and red basal half of the posterior femurs and lateral area of the thorax (Fig. 5A), while *P. chromobapta* has a blue head, a red band on the second basal quarter of the posterior femurs, yellowish-green sides of the thorax, and a black dorsal-lateral band that extends from the anterior edge of the pronotum to the tip of the tegminaes (Figure 5B).

Anatomically, *P. grilla* **sp. nov.** has a rougher integument compared to *P. chromobapta*, with deeper ~~punctures~~ punctuations on the thorax and head. The lateral carinae on the pronotum are more pronounced in the female (nymph)^[gt19] of *P. grilla* **sp. nov.**, while in the male, they are mainly visible at the posterior end, unlike the Bolivian species where they are absent. Additionally, the posterior edge of the pronotum has a slightly more angular shape in the Ecuadorian species. The frontal costa in *P. chromobapta* is sulcate, whereas, in *P. grilla* **sp. nov.**, it appears punctuated by two parallel lines of consecutive dots. The antennae ~~size~~ in *P. grilla* **sp. nov.** is slightly ~~smaller~~ shorter than the total body length, while in *P. chromobapta*, it is slightly ~~larger~~ longer. The male terminalia shape, when viewed from the ~~top~~ dorsum, is rounded in the Ecuadorian species and more angular in the Bolivian species.

In life, males of the two species of *Pseudoutanacris* typically stand upright with their front legs extended, hind legs poised to jump, and heads raised, displaying their antennae (Fig. 5A-B). In contrast, females, with more camouflaged colors, tend to adopt a flattened posture with their legs bent.

Distribution and habitat

This species is only known from the type locality in the montane forest ecosystem of the Amazonian Andean foothills of the Province of Morona Santiago, in the Ecuadorian Amazon. The collection site is a disturbed area dominated by grass, bushes, and remaining patches of forest, which are part of the buffer zone of the Sangay National Park. The exact collection point corresponds to an area of tall grass surrounded by bushes.

Behavior

In a small patch of tall grass measuring three to four square meters, we observed around a dozen red grasshoppers perched on the upper leaves of the ~~kikuyu~~ Kikuyu grass (*Cenchrus clandestinus*), an invasive species from Africa. Upon closer inspection for photography and collection, we identified two different species, *Pseudoutanacris*-*P. grilla* **sp. nov.** and *Megacheilacris graminicola* (Romaleidae), both coexisting in the same habitat. The individuals of *Pseudoutanacris* were more spread out compared to those of *Megacheilacris*. When we collected specimens, we only found male individuals of *Pseudoutanacris*, while *Megacheilacris* was represented by males, females, and juveniles. Locating *Pseudoutanacris* females was challenging, as they were well camouflaged in the lower part of the vegetation, blending in seamlessly with the dense grass. We were only able to collect one female in a juvenile state^[gt20], prompting initial doubts about whether it belonged to the same species.

This episode highlights interesting aspects of *P. grilla* **sp. nov.**: Firstly, there is the marked sexual dimorphism, which is not only related to size, shape, and color but also to the differentiated behavior between both sexes. While the males are exposed in a characteristic

raised position in the upper zone of the vegetation, the females hide among the low stems in a flattened position. A second aspect is related to communication, not only in terms of aposematism, which is present, in its own way, in both sexes, since in the case of females, these colors remain hidden until the moment of maximum danger, when they extend their legs and expose the red tibiae and the inner surfaces of the hind femurs to deter predators in the foliage. In the case of males, their colors are evident to aerial predators. However, also related to communication is the presence of antennae of considerable length, adorned with white at the tip, like a flag. This characteristic is shared with *Megacheilacris*, evidencing a probable elaborate communication mechanism in both species (Klaus Riede, pers. comm.). Finally, a third element of great interest is undoubtedly related to convergent evolution, expressed in the coloration of the males of two different groups, in which two independent species acquire similar characteristics as a survival strategy. There are undoubtedly several questions that remain that we would like to clarify in future behavioral studies; however, these are aspects that position this genus and this species as interesting subjects of study in the field of sexual evolution, adaptability, and inter and intraspecific communication.

Etymology

~~The word "grilla" is derived from the Spanish word "grillo," which refers to the female of an orthoptera species known as "grillo" (Genus *Gryllus*) and taxonomically belongs to the infraorder Gryllidea. In Ecuador, most orthoptera are commonly referred to as "grillos" (crickets). The term is also used as an Ecuadorianism to describe a person who seeks attention, exhibiting behavior similar to that of the species.~~

New geographical records

***Megacheilacris graminicola* (Descamps & Amédégnato, 1971) (Fig. 5D)**

Type Locality: Colombia, Departamento de Putumayo, entre El Mirador y pepino, altitud 1.500m, 1♂ Holotype, 1♀ Allotype, 22♂ and 18♀ paratypes, 8 larves, 8-XI-1968. Lg. M. Descamps, E. Lagos, R. Restrepo y H. Salazar. Depository: Museum of Paris.

New records: “[♂] Ecuador. Morona Santiago, / M. El Tigrillo, road Macas- / Guamote 1920 m. / -2,217458, -78,224425 / 25-ago-2023 F. Campos”; “[Depository:] MECN-FC-1700”

“[1♂, 1♀] Ecuador. Napo / Baeza, junto Río Quijos / -0.457873, -77.89381 / 17-11-2021 1800m / Manual F. Campos”; “[Depository:] MECN-FC-0094; MECN-FC-0100”

Distribution: Piemontane and Lower Montane Forest (500 to 2,000 m) between the Department of Putumayo, Colombia, to the Province of Zamora Chinchipe, in southern Ecuador.

Discussion

Our study has identified a new species of grasshopper, *Pseudoutanaeris* *P. grilla* **sp. nov.**, in the montane forests of the eastern Andes in Ecuador. This discovery expands the known distribution of the genus *Pseudoutanaeris*, previously limited to Bolivia, by more than 2,000 kilometers. The distinct coloration pattern of *P. grilla* **sp. nov.**, shared with *P. chromobapta*, underscores the uniqueness of this genus within the Amblytropidini tribe.

The co-occurrence of *P. grilla* and *Megacheilaeris* *M. graminicola* in the same habitat suggests potential ecological interactions or convergent evolutionary traits, particularly in coloration and behavior. This raises intriguing questions about the adaptive strategies and communication mechanisms of these species, warranting further investigation.

Our findings also establish new geographical records for *M. graminicola* in Ecuador, contributing to a better understanding of Orthoptera diversity in the region. Differences in sexual dimorphism and behavior between male and female *P. grilla* **sp. nov.** offer insights into their ecological roles and reproductive strategies.

However, our study is limited by a small sample size and the absence of mature female specimens, hindering a comprehensive description of sexual dimorphism and reproductive biology. Future research should focus on expanding the sample size, exploring additional habitats, and conducting detailed behavioral studies to address these gaps.

Conclusions

In conclusion, our research has identified a new species, *Pseudoutanaeris* *P. grilla* **sp. nov.**, which significantly expands the known distribution of the genus *Pseudoutanaeris*. This discovery highlights the rich biodiversity of the Ecuadorian Andes and emphasizes the importance of ongoing exploration and documentation of Orthoptera in the region.

Our findings shed light on the unique coloration and behavior of *P. grilla* **sp. nov.**, suggesting potential ecological interactions and convergent evolution with other grasshopper species. The new geographical records for *M. graminicola* further contribute to our understanding of Orthoptera diversity in Ecuador.

Future studies should focus on addressing the limitations of our research by increasing sample sizes, exploring additional habitats, and conducting comprehensive behavioral analyses. These efforts will deepen our knowledge of the ecological roles, adaptive strategies, and evolutionary relationships of these intriguing insects.

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<https://doi.org/10.1371/journal.pone.0066213>.