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Description of *Phelene reinschmidti* from Ecuador with notes on the subfamily Lophotettiginae (Orthoptera, Tetrigidae)

Niko Kasalo¹, Martin Husemann², Thomas van de Kamp^{3,4}, Josip Skejo⁵

1 Laboratory of Evolutionary Genetics, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia

2 Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstraße 13, D-76133 Karlsruhe, Germany

3 Karlsruhe Institute of Technology (KIT), Institute for Photon Science and Synchrotron Radiation (IPS), Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Karlsruhe, Germany

4 Karlsruhe Institute of Technology (KIT), Laboratory for Applications of Synchrotron Radiation, Kaiserstr. 12, D-76131 Karlsruhe, Germany

5 University of Zagreb, Faculty of Science, Department of Biology, Evolution Lab, Horvatovac 102a, HR-10000 Zagreb, Croatia

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Corresponding author: Niko Kasalo (nkasalo@irb.hr)

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Abstract

Lophotettiginae Hancock, 1909, endemic to the Neotropics, is one of the most ill-known subfamilies of Tetrigidae. Until now, there have not been any hypothesis on its relationship with other subfamilies. Lophotettiginae comprise only two genera, *Lophotettix* Hancock, 1909 and *Phelene* Bolívar, 1906, both with an arduous taxonomic history. Here, we describe a new species, *Phelene reinschmidti* from Ecuador, and compare it with its congeners: *P. turgida* from Peru and *P. maroon* from French Guiana. We found the subfamily Lophotettiginae to most closely resemble the Neotropical subfamily Metrodorinae, meaning that the two are likely related. We also provide a 3D scan of the holotype, a first for Tetrigidae. The 3D model contains all the important taxonomic characters and much more data could be extracted in the future using artificial intelligence-assisted approaches.

Key Words

3D scan, Neotropics, taxonomy, pygmy hoppers, grouse hopper

Introduction

Tetrigidae are a relatively specious orthopteran family with around 2000 described species (Cigliano et al. 2024). The family is currently undergoing revision at all taxonomic levels, with papers being regularly published on taxa in all biogeographical regions (Cigliano et al. 2024). The Neotropical region is especially understudied—only a few recent studies have introduced some brief taxonomic acts (Itrac-Bruneau and Doucet 2022, 2023; Kasalo et al. 2022, 2023).

Among the many problematic taxa, the subfamily Lophotettiginae Hancock, 1909 is perhaps the most elusive one. It comprises only two genera, *Lophotettix* Hancock, 1909 and *Phelene* Bolívar, 1906, the specimens of which are extremely rarely observed (Kasalo et al. 2022; Itrac-Bruneau and Doucet 2023). Furthermore, it is the only tetrigid subfamily endemic to the Neotropical biogeographic region. Likely, also due to its rarity, there is a complete absence of information on its position in the Tetrigidae phylogenetic tree.

The genus *Phelene* was briefly synonymized under *Chiriquia* Morse, 1900 (Cadena-Castañeda and Cardona 2015), but was quickly resurrected alongside a description of a new species, *Phelene maroon* (Cadena-Castañeda et al. 2021). The genus *Lophotettix* has already been revised to the extent that the currently available data permits (Silva et al. 2019; Kasalo et al. 2022), leaving only *Phelene* to be reevaluated in light of recent taxonomic advances.

One important step to facilitate future taxonomic revisions and make them more independent of the geographic position of the researchers is enhancing digitalization efforts. The digitalization of type specimens is of special

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importance since it allows quick and easy access to the material without risking damaging specimens and without consuming the curators' time. However, the vast majority of insect type specimens has not been photographed, and many of those that have been digitalized in this way only have dorsal views available, which severely limits their usefulness (Mertens et al. 2017; Ströbel et al. 2018). For proper taxonomic utilization, multiple views should be provided, and ideally, a scaled 3D model would be produced since it allows the specimen to be seen as it exists in reality, and more precise measurements can be taken, even without the actual specimen at hand (Ströbel et al. 2018). Unfortunately, even the simple photographic equipment is prohibitively expensive for many institutions that house type specimens, making well-photographed types rare and 3D scans nearly non-existent (Mertens et al. 2017; Ströbel et al. 2018). Until now, no 3D scans of Tetrigidae existed.

Here, we describe *Phelene reinschmidti* sp. nov., a new species of this understudied genus and provide a 3D scan of the holotype, a first among Tetrigidae. Furthermore, we review the status of the genus and provide the first hypothesis about the phylogenetic placement of Lophotettiginae.

Materials and methods

Basic photography and measurements

The specimen was examined and photographed by Mathias Vielsäcker at SMNK using a Canon EOS R5 with either a Canon EF 100MM F/2.8L MACRO IS USM for the dorsal, ventral and lateral images, or a Laowa 25 mm f/2,8 Ultra Macro 2,5–5X for the images of smaller details. Stacked imaging was performed using a WeMacro rail. Images were then stacked using Helicon Focus.

Measurements were made using ImageJ 1.53t software (Rueden et al. 2017). Taxonomy follows the OSF (Cigliano et al. 2024). Morphological terminology and measurements follow Tumbrinck (2014), with some additional measurements. The following measurements were taken: body length (BL), pronotum length (PL), pronotum width (shoulders) (PWS), pronotum width (maximal) (PWM), pronotum height (maximal) (PHM), vertex width (VW), compound eye width (CEW), antennal groove width (AGW), scutellum width (SW), fore femur length (FFL), fore femur width (FFW), mid femur length (MFL), mid femur width (MFW), hind femur length (HFL), hind femur width (HFW), tegmen length (TL), tegmen width (TW). Nomenclature is in accordance with the rules of the International Code of Zoological Nomenclature (ICZN 1999).

Synchrotron X-ray microtomography

The specimen was scanned in six height steps using a filtered polychromatic beam at the high-throughput tomography station at the Imaging Cluster of KIT Light Source. We employed an air-bearing rotary stage (RT150S, LAB Motion Systems) for sample rotation and a fast indirect detector system consisting of a 200 μ m LuAG scintillator, a double objective white beam microscope (Optique Peter; Douissard et al. 2012) and a 12-bit pco.dimax high-speed camera (Excelitas PCO GmbH) with 2016 × 2016 pixels of 11 μ m physical size. We employed a magnification of 2×, resulting in an effective pixel size of 6.11 μ m. For each scan, we took 200 dark field images, 100 flat field images, and 3000 equiangularly spaced radiographic projections in a range of 180° with an exposure time of 30 ms. The control system concert (Vogelgesang et al. 2016) served for automated data acquisition and online reconstruction of tomographic slices for data quality assurance. The 3D tomographic reconstruction was performed by tofu (Faragó et al. 2017) and additionally included ring removal and 8-bit conversion.

Post-processing of tomographic data

The individual tomographic volumes were registered and merged in Amira 2022.2, which was also employed for pre-segmentation. Pre-segmented labels served as input for semi-automatic segmentation with Biomedisa (Lösel et al. 2020). The Biomedisa result was imported back into Amira and minor errors were corrected. The needle was digitally removed and the holes filled. The final label field was converted into a polygon mesh and exported as OBJ file. CINEMA 4D R20 was employed for smoothing the surface and for polygon reduction. The polygon-reduced model was imported into MeshLab, converted into U3D format and integrated into an interactive 3D PDF document (Suppl. material 1).

Results

Taxonomy

Subfamily Lophotettiginae Hancock, 1909

Diagnosis. Frontal costa bifurcation in upper third of eye height. Antennal groove at bottom margin of eyes. Antennomeres flattened, apical one white-tipped. Vertex wider than an eye. First segment of anterior and middle tarsi a little elongated. Hind tarsus very long, first and third segment approximately equally long. Elongated slim ovipositor.

Type genus. *Lophotettix* Hancock, 1909, type species *Lophotettix brevicristatus* Hancock, 1909.

Composition and distribution. Two genera, *Lophotettix*, *Phelene*, endemic to Southern and Central America.

Genus Phelene Bolívar, 1906

- Nephele Bolívar, 1887: 252. (junior homonym of Nephele Hübner, 1819);
- Phelene Bolívar, 1906: 392. (replacement name); Kirby 1910: 23. (catalogued); Günther, 1938: 307. (diagnosis); Yin et al. 1996: 899.

(catalogued); Cadena-Castañeda and Cardona 2015: 477. (noted as a synonym of *Chiriquia*); Silva et al. 2019 (mentioned); Cadena-Castañeda et al. 2021: 357. (revision);

Gladiotettix Hancock, 1907: 40. (diagnosis in English, obsolete replacement name); Bruner 1910: 101. (Hancock's descriptions of the species which was later assigned to *Lophotettix*).

Differential diagnosis. Antennal segments flattened, but oval (apical segments triangular in *Lophotettix*). Low pronotal crest (medium to tall in *Lophotettix*). Anterior and middle femora smooth (moderately tuberculated in *Lophotettix*).

Type species. Phelene turgida (Bolívar, 1887)

Composition and distribution. *P. maroon* Cadena-Castañeda & Tumbrinck, 2021, *P. turgida* (Bolívar, 1887), *P. reinschmidti* sp. nov. The genus seems to be restricted to the northern half of South America.

Note. We identified two female specimens of *P. turgida* from Peru at the Zoologische Staatssammlung München (ZSM), which we present in Fig. 1 to aid in comparison with the new species. Additionally, we identified two male specimens of this species at the ZSM with the following label data: Peru, Prov. Huanuco / b. Yuyapichis, Stat. Panguana / 9°37'S, 74°36'W Lux Wald / Baum liegend, 20.9.–7.10.2013 / leg. Burmeister.

Phelene reinschmidti Kasalo, Husemann & Skejo, sp. nov.

https://zoobank.org/6047D7A1-7F3F-40FF-BDD2-4E2B67592A16 Fig. 2

Diagnosis. Easily separated from the congeners by the following set of characters: (i) median carina forming a low elongated crest which extends from between the prozonal carinae to the base of hind legs; (ii) vertex a little less than two eyes wide; (iii) brown body without distinct patterns; (iv) rounded pulvilli of hind tarsi; (v) female subgenital plate oval with moderately protruding triangular apex; (vi) alae dark brown with venation of the same color. Differs from *P. maroon* by characters iii, iv, v, and vi.

Description. Macropronotal. In anterior view, top of vertex approximately at level of upper margin of eyes. Vertex a little less than two eyes wide. Frontal costa bifurcation in upper third of eye height. Facial carinae forming long oval shape. Paired ocelli approximately at

half of eye height. Midline of antennal groove at level of bottom margin of eyes. Antennae composed of 13 visible segments; apical six flattened, last segment white. In dorsal view, vertex of isosceles trapezoidal shape. Medial carina visible in anterior half of vertex. Lateral carinae in form of small tubercles. Prozonal carinae as long as eye as seen in dorsal view, strongly convergent caudad. Median carina visible throughout length of pronotum, forming low but long crest in lateral view (extending from between prozonal carinae to base of hind legs). Pronotal surface covered in small tubercles and carinulae. Humeral angles wide and blunt. Lateral lobes rectangular, projected laterally, with thin white border. Tegmina large and oval. Alae reaching pronotal apex; dark brown with dark brown venation. Anterior and middle femora long and smooth. Anterior and middle femora long and smooth. Anterior and middle tarsi with long first segment and extremely long second segment. Hind femur robust, with two tubercles on dorsal margin; moderately sized sharp antegenicular tooth, genicular tooth barely visible. Hind tibia thin, serrated along caudal margin. Hind tarsus long; first segment extremely long, with three elongated pulvilli; third segment missing in the holotype. Ovipositor extremely elongated, serrated. Base of subgenital plate with triangular notch; shallow stitch line extending from it throughout most of the length of the plate. Subgenital plate elongated with moderately protruding triangular apex.

Measurements (in mm). BL (9.21); PL (12.33); PWS (2.19); PWM (2.85); PHM (2.38); VW (0.84); CEW (0.52); AGW (0.23); SW (0.3); FFL (2.16); FFW (0.45); MFL (2.28); MFW (0.49); HFL (4.75); HFW (1.58); TL (1.47); TW (0.58)

Holotype. Adult female collected in early 1993 by Duffner in Ecuador, Sucumbios Province on the upper Aguarico. The holotype is deposited at the SMNK (Karlsruhe, Germany). See Fig. 2 and Suppl. material 1.

Exact label information. "ECUADOR (Sucumbios) / Oberer Aguarico, Anfang / 1993 leg. Duffner // 101, // Staatl. Museum / für Naturkunde / Karlsruhe; / ex coll. Riede, / Inv. Nr. E-Orthop-2"

Terra typica. Ecuador, Sucumbíos Province. No precise locality known.

Distribution. Known only from a single specimen.

Etymology. The new species is named after Prof. Dr. Matthias Reinschmidt, the current director of the Karlsruhe Zoo for his dedication and investments in nature protection in Ecuador and around the world.

Identification key to the species of Phelene

1	Tall narrow pronotal crest above tegmina	Phelene turgida
_	Low elongated pronotal crest	
2	Lower half of body distinctly black, upper half black or brown; pulvilli of hind tarsus triangular and sharp; alae dark with	
	bright venation; female subgenital plate oval with slight tubercle at its apex	Phelene maroon
_	Body uniformly brown; pulvilli of hind tarsus rounded and blunt; alae and their venation	n dark brown; female subgenital
	plate oval with moderately protruding triangular apex	Phelene reinschmidti sp. nov.



Figure 1. *Phelene turgida* specimens deposited in ZSM. A. The locality where the specimens were collected; B. Label, same for both specimens; C. Two female specimens depicted in lateral, frontal, and dorsal views. All photos are to the same scale except the frontal views. Scale bar: 1mm.

Discussion

General remarks

The new species is at first glance quite similar to *Phelene maroon*. The latter was until recently known only from a single male specimen (Cadena-Castañeda et al. 2021), but a recent examination of collections from

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French Guiana revealed a number of new individuals, of which many were female (Itrac-Bruneau et al. 2023). This, coupled with our own *P. turgida* specimens, allowed us to thoroughly compare our only female specimen with *P. maroon* females and find some key differences, most notably in the shape of the subgenital plate. Furthermore, *P. reinschmidti* sp. nov. was collected in Ecuador, while *P. maroon* is known only from French



Figure 2. *Phelene reinschmidti* sp. nov. A. Regions where *Phelene* species have been found, red shapes represent type localities, star–*P. reinschmidti*, circle–*P. turgida*, triangle–*P. maroon*; B. Frontal view; C. Dorsal view; D. Ventral view; E. Abdominal apex in ventral view; F. Lateral view; G. Image of the 3D model we generated.

Guiana. Interestingly, *P. turgida* is known from both Peru and French Guiana, and is sometimes syntopical with *P. maroon*. The specimens of *P. turgida* reported by Itrac-Bruneau et al. (2023) differ from the specimen from Marcapata, Peru by the shape of the pronotal crest and may represent a separate species. However, our specimens collected in central Peru (Fig. 1) exhibit the same amount of variability in this trait as between the French Guiana and Marcapata specimens, indicating either that this trait can vary somewhat or that the distribution of *Phelene* species is very complex. Unfortunately, photographs of the *P. turgida* types are not available. It is still possible to describe tetrigid species based on clear morphological characters, but DNA studies are becoming increasingly necessary, especially to address interesting biogeographical observations.

Phylogenetic hypotheses

Until now, the question of the position of Lophotettiginae in the Tetrigidae phylogeny remained completely unaddressed. The brief synonymization of *Phelene* with *Chiriquia* (Cadena-Castañeda and Cardona 2015) was never elaborated despite being interesting and potentially premonitory. These two genera are clearly not synonymous, but their similarities cannot simply be explained as convergence (Cadena-Castañeda et al. 2021). *Phelene* and *Chiriquia* are alike in their facial morphologies, lengths of first tarsal segments, the placement of pronotal crests, and the sideways projected lateral lobes, which could point to some degree of relatedness. The genus *Otumba* Morse, 1900 seems to be related to *Chiriquia* and could provide further hints upon its urgently needed revision.

Further, we have to consider the genus *Metrodora* Bolívar, 1887. Some of its species, e.g., *M. lutosa* Bolívar, 1887, *M. gibbinota* (Bruner, 1910), and *M. panamae* (Hebard, 1924) also exhibit the earlier listed similarities with *Phelene*, while *M. gibbinota* and *M. panamae* also have white-tipped antennae. This genus and by extension its tribe, Metrodorini Bolívar, 1887, is composed of likely related but ill-defined taxa, which is hindering further research (Kasalo et al. 2023). The relationship between Metrodorinae and Lophotettiginae should be closely examined and it may even find the latter to be synonymous with the former.

Lastly, all these genera share the mentioned characters with many members of the predominantly African tribe Xerophyllini Günther, 1979, currently attributed to the subfamily Cladonotinae. Therefore, the possibility that Lophotettiginae, Metrodorinae, and Xerophyllini form a monophyletic group should be seriously considered and examined from both morphological and molecular angles. The morphology akin to *Morphopus* (Bolívar, 1887) (Xerophyllini) could be basal in this group, which could explain all the different derived morphologies within it, as well as its distribution across the southern half of Gondwana. It should be noted that we do not consider Asian Metrodorinae as true members of that subfamily and that all of the mentioned higher taxa require extensive revisions.

Value of 3D data in taxonomy

We here provide the first 3D data for any Tetrigidae, though more are in preparation. Such 3D data may become an important resource in future taxonomic works, especially in concert with artificial intelligence and machine learning approaches. One of the large advantages of including 3D data in taxonomic descriptions is that virtual specimens will be readily available around the world, without the danger of sending valuable and irreplaceable holotypes and risking them getting lost or damaged. In addition, such virtual specimens are at least a minor backup in case a type gets lost. The 3D models are scaled to size and allow easy and accurate measurement of distances and also have the benefit of allowing for 3D geometric morphometric measurements. Modern high-throughput X-ray imaging methods already offer the possibility to digitize large numbers of insects in a short time (van de Kamp et al. 2018; Rühr et al. 2021). In the future, artificial intelligence will help to further automate data acquisition as well as large-scale data analysis (Heethoff and van de Kamp 2023). Even statistical species delimitation may become a viable approach potentially speeding up the process of taxonomy, an important improvement in a time of heavy diversity decline.

Author contributions

NK: first and last draft, taxonomy, measurements, figures; MH: last draft, specimen handling; TvdK: last draft, 3D digitalization; JS: last draft, taxonomy.

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Supplementary material 1

3D reconstruction of *Phelene reinschmidti* sp. nov.

Authors: Thomas van de Kamp

Data type: pdf

- Explanation note: 3D reconstruction of *Phelene rein-schmidti* sp. nov. Click on the image to start the interactive 3D view; use the menu to switch between the standard views.
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