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Article *in* Proceedings of the Entomological Society of Washington · July 2024

DOI: 10.4289/0013-8797.125.4.493

1 author:

Jason L. Williams
University of Florida

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Author: Williams, Jason L.

Source: Proceedings of the Entomological Society of Washington, 125(4) : 493-497

Published By: Entomological Society of Washington

URL: https://doi.org/10.4289/0013-8797.125.4.493

PROC. ENTOMOL. SOC. WASH.
125(4), 2023, pp. 493–497

HIGH-RESOLUTION NANO-COMPUTED TOMOGRAPHY OF AN ASYMMETRIC GENITAL DUPLICATION EVENT IN A *NYLANDERIA FULVA* (MAYR) (HYMENOPTERA: FORMICIDAE) MALE

Jason L. Williams

(JLW) Entomology and Nematology Department, University of Florida, Gainesville, FL, USA (e-mail: jleewill@gmail.com, urn:lsid:zoobank.org:author:3EE27CAE-1684-41C4-A550-DFF104B27A9C, https://orcid.org/0000-0002-9834-3348)

Abstract.—Teratology in arthropods is widely reported, but the mechanisms leading to abnormal development are under-studied. This study focuses on a teratological case discovered during dissection, in which a male of the tawny crazy ant (*Nylanderia fulva*) exhibited genital duplication. Using nano-computed tomography, I generated three-dimensional models of the genital capsule to describe which structures were duplicated. The left side of the genital capsule appeared as normal, but the right side exhibited anomalies such as two additional gonostyles (parameres), an extra gonossiculus (digitus) on the volsella, and an additional volsella-like growth bearing peg-like denticles. This growth emerged between the mediad extra gonostyle and the penisvalva.

Key Words: abnormality, morphology, mutation, nano-CT

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Teratology is the scientific discipline dedicated to examining the causes, mechanisms, and consequences of aberrant development in organisms, with a focus on understanding the influences of factors such as genetic mutations and environmental stressors (Calado and Pires 2018). Supernumerary and deformed appendages are known to occur across Arthropoda, having been reported in Acari, (Chitimia-Dobler et al. 2017, Eeva and Penttinen 2009), Araneae (Izquierdo 2020), Branchiopoda (Miličić et al. 2013), Decapoda (Spanò et al. 2003), Opiliones (Kozel and Novak 2013), and Pycnogonida (Scholtz and Brenneis 2016) In insects, these anomalies have been reported in Coleoptera (Asiain and Márquez 2009, Balazuc 1948, Frank 1981), Diptera (Voorhees and Horsfall 1971), Hymenoptera (Balazuc 1958, Gülmez 2019, Mariano et al. 2022), and Lepidoptera (Balazuc and Bourgogne 1969, Balazuc and Pointel 1956, Hayden 2019).

The tawny crazy ant, *Nylanderia fulva* (Mayr 1862) (Fig. 1), is an invasive ant species from central South America. The earliest records of this species in the United States are from Texas in 1938 (Trager 1984), but it was not considered a serious pest until the early 1990s (Klotz et al. 1995). Since then, it has become established across all Gulf Coast states, from Texas to Florida (Meyers and Gold 2008).

Figs. 1, 2. *Nylanderia fulva*. 1, Habitus of a male in profile view. Specimen shares locality and collection data with the mutant male. Image taken by Helen-Rose Beiriger. Scale bar 1.0 mm. 2, Genital capsule of the mutant specimen in dorsal view. Scale bar 0.1 mm.

Presently, the only diagnostic morphological characters to identify this species are found on the male genitalia (Gotzek et al. 2012). While practicing dissection of male *N. fulva* genitalia in December 2021, I discovered an individual with numerous additional structures that had developed from the right side of the genital capsule (Fig. 2). To further investigate and characterize this phenomenon, I decided to nano-CT scan and examine the genital capsule more closely.

Materials and Methods

Locality information.—The specimen was taken from a nest series collected in Iowa Colony, Texas (coll. E. LeBrun, 17 Nov. 2009; GPS coordinates: 29.43 d. lat., -95.44 d. long.) and is deposited in the Smithsonian Institution National Museum of Natural History Insect Collection (USNM).

Dissection.—A 10% potassium hydroxide (KOH) solution was brought to just below boil in a glass dish on a hot plate. The metasoma was removed, placed in the KOH solution for clearing, checked every five minutes, and removed from solution once cleared (~ 10 minutes). The cleared metasoma was rinsed in a dish of 95% ethanol and then transferred to a small watch glass with a drop of 99.5% anhydrous glycerin, where the genital capsule was delicately excised from the rest of the metasoma using two minuten probes.

Nano-CT scanning and segmentation.—The genital capsule was mounted on a paper point and scanned using a Zeiss Versa 620 XRM scanner with a tungsten target (binning = 1, magnification = 20x, voltage = 80 kV, current = 125 μA, voxel size = 0.2607 μm) at the University of Florida Nanoscale Research Facility. Segmentation was completed using Dragonfly ORS (version 2022.2). Three-dimensional models were exported as .stl files.

Results

Terminology in the following description is adapted from Dal Pos et al. (2023). Upon closer examination, the left side of the genital capsule appeared as normal for the species, but the right side exhibited supernumerary gonostyles, an additional gonossiculus on the volsella (Fig. 3), and one additional volsella-like growth (bearing the same peg-like denticles normally seen on the surfaces of the gonossiculus and cuspus) budding from between the mediad extra gonostyle and the penisvalva.

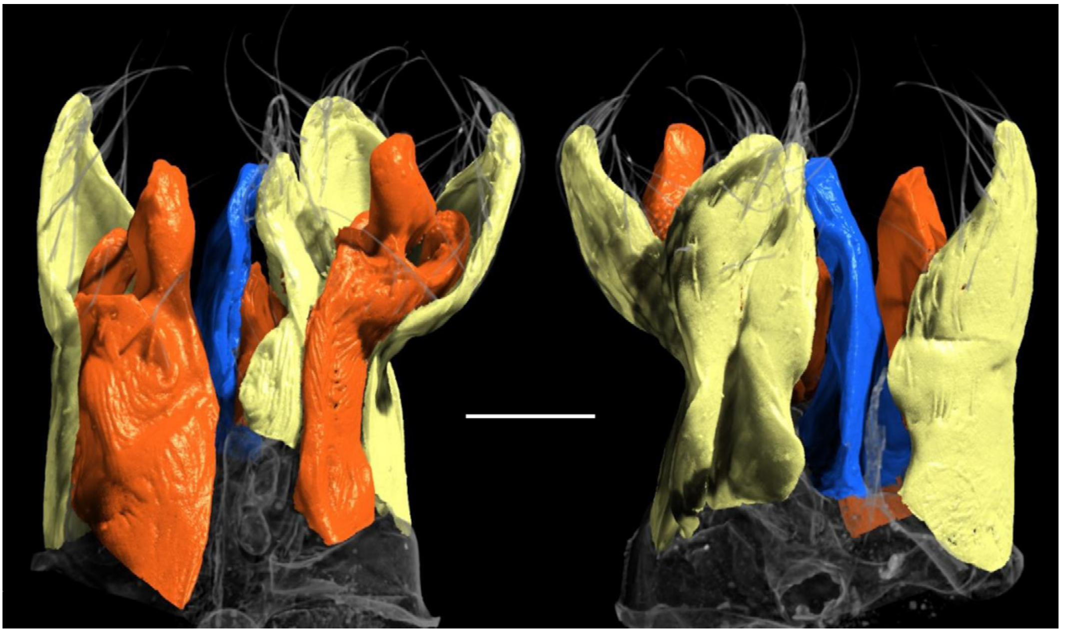

Fig. 3. Genital capsule of the mutant *Nylanderia fulva* in ventral (left) and dorsal (right) views via nano-CT scan. Areas colored in yellow are gonostyles, those in orange are volsella, and those in blue are the penisvalva. Scale bar 100 µm.

Additionally, the sclerites of the penisvalva were asymmetrical in shape. The rest of the specimen, including the right side of the genital capsule, appeared as normal. All other males collected from the same nest series (n = 12) appeared as normal, without any mutations.

Discussion

By bringing to light this case of supernumerary structures in a *N. fulva* male, I hope to bring increased attention to the study of teratological phenomena for understanding underlying developmental processes. Teratological phenomena are widely reported across Arthropoda, but in most cases their underlying mechanisms are under-studied. Causes include genetic mutations and environmental factors, among others. Hox genes are often cited as genetic factors, especially in supernumerary or abnormal appendages (Denell et al. 1981, Hughes and Kaufman 2002). However, environmental stressors such as cytotoxins (Buczek et al. 2019), chemical pollution (López Greco et al. 2001), temperature and light (Napiórkowska et al. 2018), and parasitism and predation (Peltzer et al. 2011) have also been implicated in abnormal development.

Although nano-CT allows for high-resolution capture of internal and external structures, it sometimes generates artifacts and is not always capable of fully resolving connections between sclerites, conjunctiva, and muscles. To circumvent these issues, Dal Pos et al. (2023) strongly advise visualizing these structures in glycerol without allowing them to dry and imaging via confocal laser scanning microscopy. Therefore, future studies of arthropod genitalia should follow these recommendations to provide a more comprehensive understanding of skeletomusculature arrangement and a more nuanced inference of anatomical ontology and homology.

Acknowledgments

Thanks go to Ed LeBrun for collecting the specimen, John S. LaPolla for aiding with dissection, and James Hayden for encouraging me to publish about this unusual specimen. I also thank Aswaj Punnath for nano-CT scanning assistance, and Ed Stanley and Gary Schieffele at the University of Florida Nanoscale Research Facility for providing technical support with the Nano-CT equipment. Finally, I would also like to thank Helen-Rose Beiriger for taking the *Nylanderia fulva* male profile image.

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