



## RESEARCH ARTICLE - ANTS

## First Karyotype Description of *Acanthognathus rudis* Brown & Kempf, 1969 (Formicidae: Myrmicinae) with Notes on Its Natural History

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### Abstract

The trap-jaw ant *Acanthognathus* Mayr, 1887 (Formicidae: Myrmicinae) has been subject of various studies since 1969, but its cytogenetics remains unexplored. This study provides the first karyotype description of a representative of this genus, *A. rudis*, including chromosomal number, morphology, and the distribution of repetitive sequences. A colony collected in Southeastern Brazil exhibited a diploid number of  $2n = 14$  (12 metacentrics + 2 submetacentrics), the lowest number among the *Daceton* genus-group and the first Neotropical representative of this group to be karyologically studied. The rDNA clusters were located in the pericentromeric region of a single chromosome pair,  $(GA)_n$  blocks cover the entire chromosome arms except for heterochromatic regions, and  $(TTAGG)_n$  hybridized with the telomeres of all chromosomes, without interstitial telomeric sites. The repetitive sequence patterns align with those observed in other Neotropical Formicidae. Additionally, we documented new behavioral aspects of mandible use in *A. rudis* workers and provided the first images of its immature stages. These findings emphasize the need for further research on *Acanthognathus* and the other *Daceton* genus-group.

### Introduction

The small genus of tiny trap-jaw ants, *Acanthognathus* Mayr, 1887 (Myrmicinae) is characterized by a unique mandibular locking mechanism that relies mostly on a hypertrophied basimandibular process. In this mechanism, the labrum is strongly reduced and does not play a role in its function (Bolton, 1998; Gronenberg, 1998; Baroni Urbani & De Andrade, 2007). The genus is distributed from southern Mexico to the southernmost state of Brazil, Rio Grande do Sul (Brown & Kempf, 1969; Janicki, 2016). Recent molecular phylogenetic studies have placed *Acanthognathus* within a monophyletic group of Attini referred to as “Dacatina” by Branstetter et al. (2017). Other genera composing this group include the Neotropical *Daceton* Perty, 1833 and *Lenomyrmex* Fernández and Palacio, 1999, the Afrotropical *Microdaceton* Santschi, 1913, and the Australian *Orectognathus* Smith, 1853,

*Colobostruma* Wheeler, 1927, *Epopostruma* Forel, 1895, as well as *Mesostruma* Brown, 1948 (Branstetter et al., 2017). Although Branstetter et al. (2017) refer to “Dacatina” as a subtribe, it is not a valid taxonomic subtribe name (Bolton, 2024). Therefore, we will refer to these genera collectively as the “*Daceton* genus-group”.

*Acanthognathus* underwent a comprehensive revision in 1969 by Brown and Kempf, who described three new species and provided a taxonomic history and a key for the six species known at that time. Among the newly described species was *Acanthognathus rudis* Brown and Kempf, 1969, whose immature stages were also studied in that same year (Wheeler & Wheeler, 1969). Following this revision, several studies of the genus have been published. These studies include notes on the natural history and behavior of *A. rudis* (Dietz & Brandão, 1993), the description of *Acanthognathus poinari* Baroni Urbani, 1994, a fossil species from the Dominican Amber



(Baroni Urbani & De Andrade, 1994), the catching mechanism of *Acanthognathus* (Gronenberg et al., 1998), a new revision that included the fossil species and an updated diagnosis for the genus (Bolton, 2000), a morphology-based phylogeny that included the *Daceton* genus-group and other Attini genera (Baroni Urbani & De Andrade, 2007), the description of *Acanthognathus laevigatus* by Galvis and Fernandez, 2009, which included an updated key for the genus, the first report of ergatoids in *A. rudis*, *Acanthognathus brevicornis* Smith, 1944, and *Acanthognathus ocellatus* Mayr, 1887 (Silva & Brandão, 2014), and the molecular studies that revealed the phylogenetic position of the *Daceton* genus-group within the Attini, as well as its internal organization (Ward, 2015; Branstetter et al., 2017). Despite these contributions, there is currently no data available on the cytogenetics of the genus.

Cytogenetic studies employing different banding techniques and fluorescent *in situ* hybridization (FISH) have advanced our understanding of karyotype characterization in Formicidae (Lorite & Palomeque, 2010; Cristiano et al., 2013; Teixeira et al., 2021; Barros et al., 2021, 2022; Jacintho et al., 2023). The other genera in the *Daceton* genus-group have only a few cytogenetic records, which exist solely for Australian members of the group, including *Epopostruma* sp. ( $2n = 20$ ), *Orectognathus clarki* Brown, 1953 ( $2n = 30$ ), *Orectognathus darlingtoni* Taylor, 1977 ( $2n = 22$ ), *Orectognathus versicolor* Donisthorpe, 1940 ( $2n = 22$ ), *Colobostruma alinodis* (Forel, 1913), and *Colobostruma* sp. (both with  $2n = 22$ ) (Crozier, 1968; Imai et al., 1977). As the cytogenetic research on ants requires the collection of immature developmental stages (Imai et al., 1988), this offers researchers additional opportunities to gather information on the species' natural history, thereby providing valuable insights for taxonomy, ecology, evolution, and conservation (Lopes et al., 2022; Ulysséa et al., 2024).

In this study, we conducted the first cytogenetic analysis of *Acanthognathus*, examining chromosomal number and morphology, as well as the location of 18S ribosomal genes, the microsatellite (GA)<sub>n</sub>, and telomere motif (TTAGG)<sub>n</sub> in *A. rudis*. Additionally, we included a series of observations on the ant's behavior, such as workers' foraging activities, brood care, and provided the first images of immature stages, thereby complementing the description by Wheeler and Wheeler (1969).

## Materials and Methods

One colony of *A. rudis* was collected in the Estação de Pesquisa, Treinamento e Educação Ambiental (EPTEA) Mata do Paraíso (-20.801, -42.866), an Atlantic Forest fragment located in the municipality of Viçosa, Minas Gerais State, Brazil. The colony was found in a cavity within a large decomposing log and comprised five post-*meconium* elimination larvae (pre-pupae), about five young larvae (still bearing *meconium*), three female pupae with darkened eyes,

over ten workers, one queen, and no males. The nest was carefully transferred to the Laboratory of Insect Cytogenetics at the Federal University of Viçosa (UFV), Minas Gerais, Brazil. The nest was collected on September 20, 2023, and remained alive for two weeks.

The species was identified using the keys provided by Galvis and Fernandez (2009) (modified from Brown & Kempf, 1969). Two workers (ANTWEB1053042, ANTWEB1053043) and the queen (ANTWEB1053044) were pinned and deposited in the Coleção Entomológica do Laboratório de Coleoptera (CELC, collection abbreviation follows Evenhuis, 2024) at UFV, Brazil.

Images of pinned specimens were captured using a Leica MC170 HD camera coupled to a Leica M205. Images of the young larvae were obtained using a Canon 1100D camera coupled to an Olympus CX41 Microscope with dark-field illumination. The acquired images were processed using Zerene software (Zerene Systems LLC), GIMP v 2.10.38, and ImageJ (Schneider et al., 2012). These images were uploaded to AntWeb.org. Images of living colonies were captured using a Canon 1100D with the reversed lens technique for macrophotography.

Mitotic chromosomes were obtained of four female pre-pupae and three female young larvae - still with *meconium*. The ganglia were submitted to hypotonic colchicine solution, and fixatives according to Imai et al. (1988), and then stained with 4% Giemsa. As demonstrated by Imai et al. (1988), Giemsa staining can detect heterochromatic patterns in low condensed chromosomes and was therefore employed to study the heterochromatin distribution in *A. rudis*.

Chromosomal mapping of three repetitive sequences was performed on 15 metaphases using a FISH assay. The assay followed the protocol proposed by Pinkel et al. (1986), with modifications suggested by Teixeira et al. (2022). The microsatellite (GA)<sub>n</sub> and telomere motif (TTAGG)<sub>n</sub> were used as probes directly labeled with Cyanine-3 (Cy3) at the 5' end (Sigma, St. Louis, MO, USA).

The 18S rDNA probe was produced through polymerase chain reaction (PCR) using the primers 18SF1 (5'-GTC ATA GCT TTG TCT CAA AGA-3') and 18SR1.1 (5'-CGC AAA TGA AAC TTT AAT CT-3'), which were initially designed for the stingless bee *Melipona quinquefasciata* Lepelletier, 1836 (Pereira, 2006). Genomic DNA from the ant *Camponotus rufipes* (Fabricius, 1775) was used as a template. This probe was then indirectly labeled with digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany). FISH signals were detected using a rhodamine-conjugated antibody against digoxigenin (Roche Applied Science), following the manufacturer's instructions.

Chromosomes stained with Giemsa were observed and photographed using a Q-Color3 Olympus capture system coupled to an Olympus BX60 microscope. Metaphases submitted to FISH were analyzed and photographed using an Olympus BX53F epifluorescent microscope coupled with an

Olympus MX10 camera, and images were captured using the CellSens software. Chromosome pairing was performed using GIMP v 2.10.38 software. Measurements for the short (p) and long (q) chromosomal arms, as well as the total length (TL = p + q), were obtained using the computer program Image-Pro Plus (Media Cybernetics Ltd.). Chromosomes were arranged in decreasing size based on the ratio of chromosome arm lengths ( $r = q/p$ ), and classified according to Levan et al. (1964).

All cytogenetic analyses, behavioral observations, and larval studies were conducted at the Laboratory of Insect Cytogenetics.

## Results and Discussion

### Cytogenetics

This study represents the first cytogenetic investigation conducted on *Acanthognathus* and on a Neotropical representative of *Daceton* genus-group. Analysis of 76 metaphases from *A. rudis* revealed a diploid chromosome number of  $2n = 14$ , with a karyotypic formula of  $2n = 12m + 2sm$  (Fig 1a; Table 1). Notably, the chromosome number  $2n = 14$  in *A. rudis* differs from those reported in Australian members of *Daceton* genus-group, which range from  $2n = 20$  to  $2n = 30$  chromosomes (Crozier, 1968; Imai et al., 1977).

**Table 1** Karyomorphometric analysis of the ant *Acanthognathus rudis*  $2n = 14$ . Abbreviations: **m**. metacentric; **sm**. submetacentric.

Chromosome	Chromosome arm <sup>a</sup>		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	2.29 ± 0.43	3.26 ± 0.61	5.55 ± 0.95	9.58	1.43 ± 0.20	<b>m</b>
1	2.18 ± 0.50	3.12 ± 0.43	5.30 ± 0.86	9.15	1.47 ± 0.19	<b>m</b>
2	2.01 ± 0.59	2.75 ± 0.58	4.76 ± 0.95	8.22	1.49 ± 0.75	<b>m</b>
2	1.97 ± 0.50	2.76 ± 0.55	4.73 ± 0.90	8.17	1.47 ± 0.47	<b>m</b>
3	2.07 ± 0.37	2.56 ± 0.51	4.63 ± 0.79	7.99	1.25 ± 0.21	<b>m</b>
3	2.02 ± 0.41	2.54 ± 0.45	4.56 ± 0.78	7.87	1.28 ± 0.21	<b>m</b>
4	1.91 ± 0.38	2.53 ± 0.47	4.44 ± 0.79	7.67	1.35 ± 0.20	<b>m</b>
4	1.85 ± 0.46	2.39 ± 0.49	4.24 ± 0.86	7.32	1.32 ± 0.26	<b>m</b>
5	1.19 ± 0.18	1.99 ± 0.41	3.18 ± 0.55	5.49	1.67 ± 0.27	<b>m</b>
5	1.21 ± 0.24	1.98 ± 0.46	3.19 ± 0.69	5.51	1.63 ± 0.12	<b>m</b>
6	0.98 ± 0.19	1.35 ± 0.24	2.33 ± 0.40	4.02	1.39 ± 0.20	<b>m</b>
6	0.99 ± 0.19	1.36 ± 0.33	2.35 ± 0.40	4.06	1.38 ± 0.20	<b>m</b>
7	1.23 ± 0.23	2.82 ± 0.62	4.68 ± 1.26	8.08	2.31 ± 0.44	<b>sm</b>
7	1.19 ± 0.23	2.79 ± 0.61	3.98 ± 0.79	6.87	2.37 ± 0.33	<b>sm</b>
Total			57.92	100		

<sup>a</sup>Chromosome length obtained in micrometers (μm) from 15 metaphases.

To date, *A. rudis* has the lowest chromosome number within the *Daceton* genus-group.

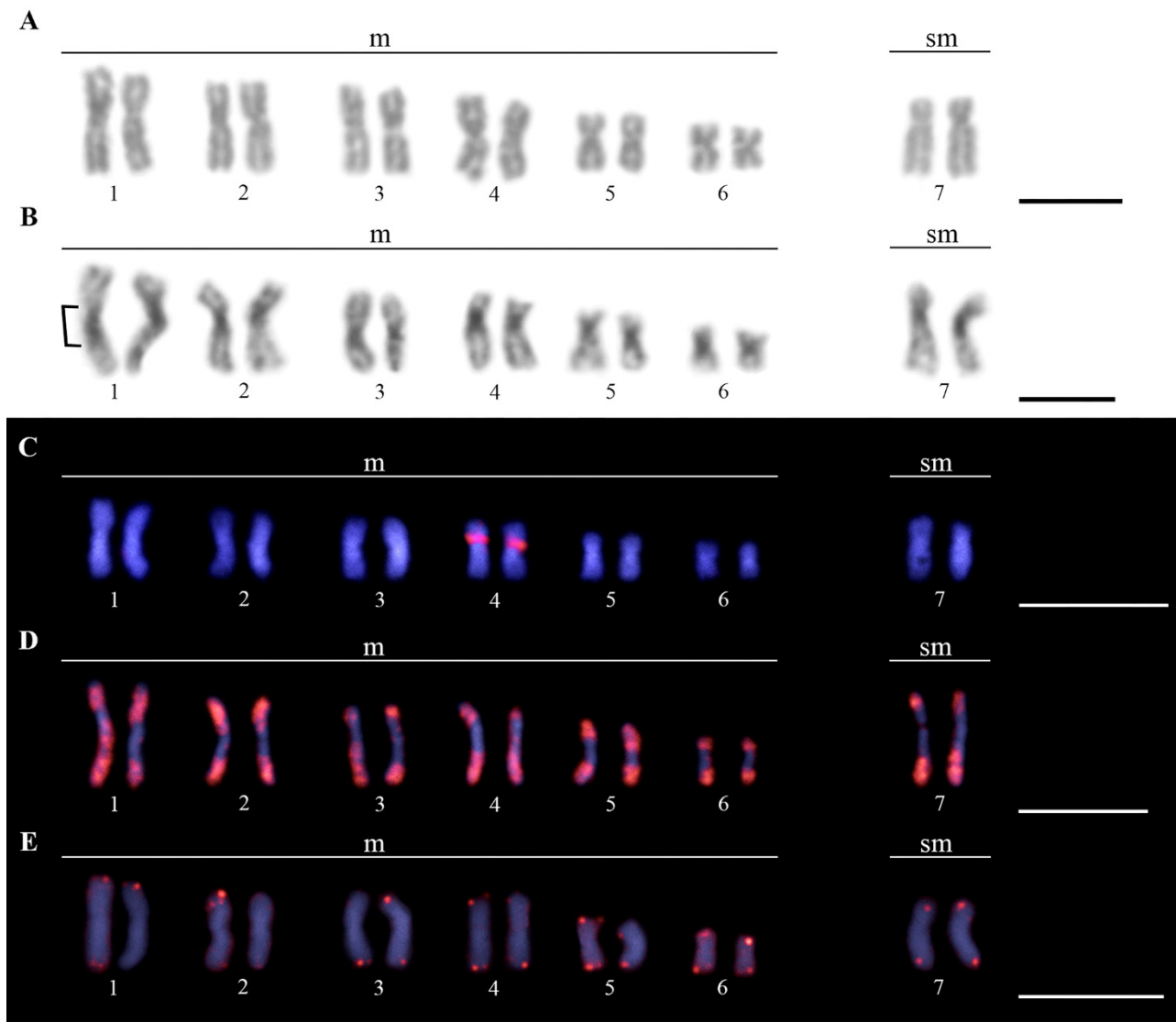
The 18S rDNA clusters were localized in the pericentromeric region of the short arms of the 4<sup>th</sup> metacentric chromosome pair (Fig 1c). This intrachromosomal location in a single chromosome pair is consistent with the prevalent pattern found in Neotropical Formicidae (Teixeira et al., 2021; Damasceno et al., 2024).

Heterochromatin, visualized through Giemsa staining, exhibited a centromeric distribution across all chromosome pairs, with euchromatin confined to the remaining regions of chromosomes (Fig 1b). The microsatellite (GA)<sub>n</sub> was distributed along the whole chromosome arms, except for the centromeric regions of the karyotype of *A. rudis* (Fig 1d), indicating that this microsatellite is localized solely in the euchromatic regions of the karyotype. The presence of (GA)<sub>n</sub>

microsatellites in euchromatic regions is a common pattern in ant species (Teixeira et al., 2022), consistent with our observations.

The telomeric motif (TTAGG)<sub>n</sub> showed hybridization signals at the telomeres of all chromosomes of *A. rudis* (Fig 1e), with no interstitial telomeric sites detected. This telomeric motif does represent an ancestral feature in Hymenoptera, as well as in insects, which apparently have been lost in the basal Apocrita and then reappearing multiple times in various families, including Formicidae (Kuznetsova et al., 2020).

As our study represents the only known report of cytogenetic data for *Acanthognathus*, direct comparisons with closely related species are currently not possible. Further research on this genus is expected to improve our understanding of its chromosomal evolution and provide insights into the phylogenetic relationships within the *Daceton* genus-group.



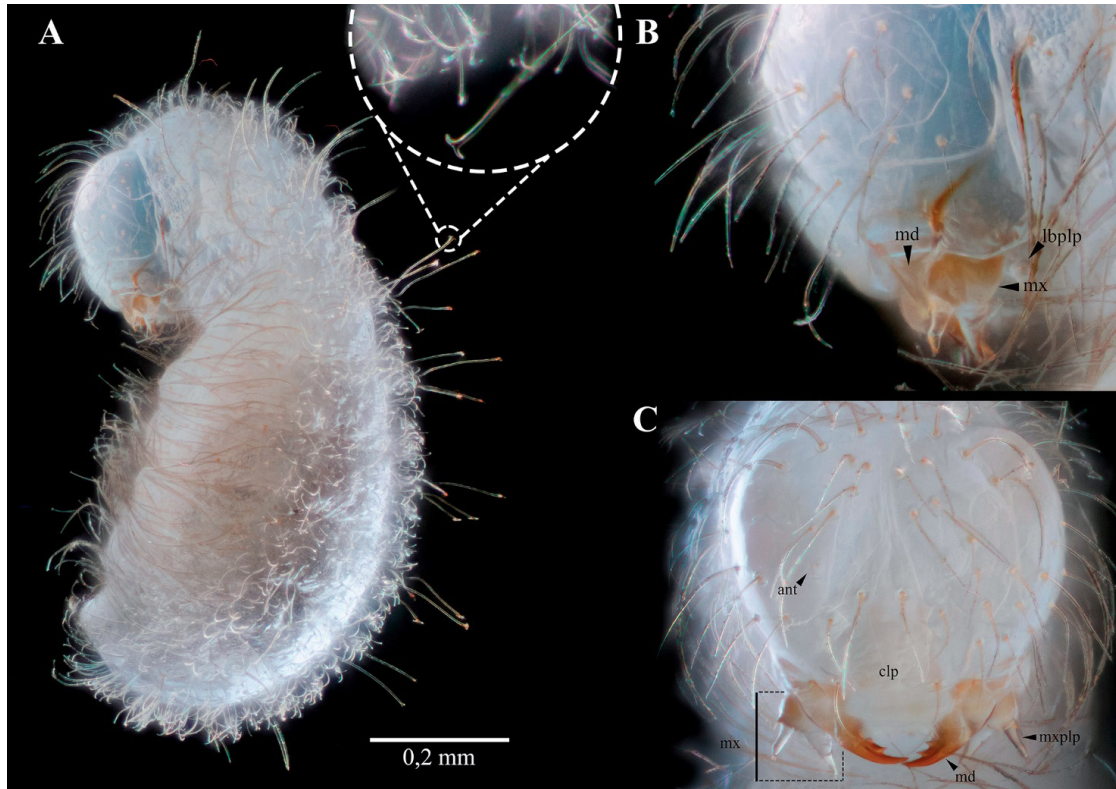
**Fig 1.** Karyotypes of *Acanthognathus rudis* ( $2n = 14$ ): **A.** Giemsa-stained karyotype of a female; **B.** Heterochromatic patterns visualized with Giemsa staining, highlighting the heterochromatic regions (dark centromeric blocks); **C.** FISH with 18S rDNA probe located in the pericentromeric region of the short arms of the 4<sup>th</sup> chromosome pair; **D.** FISH with  $(GA)_n$  probe distributed across the entire chromosomes except for the heterochromatic regions; **E.** FISH with  $(TTAGG)_n$  probe in terminal localization. Hybridization signals are shown in red. Abbreviations: **m.** metacentric; **sm.** submetacentric. Scale bars: 5 $\mu$ m.

### Biological notes

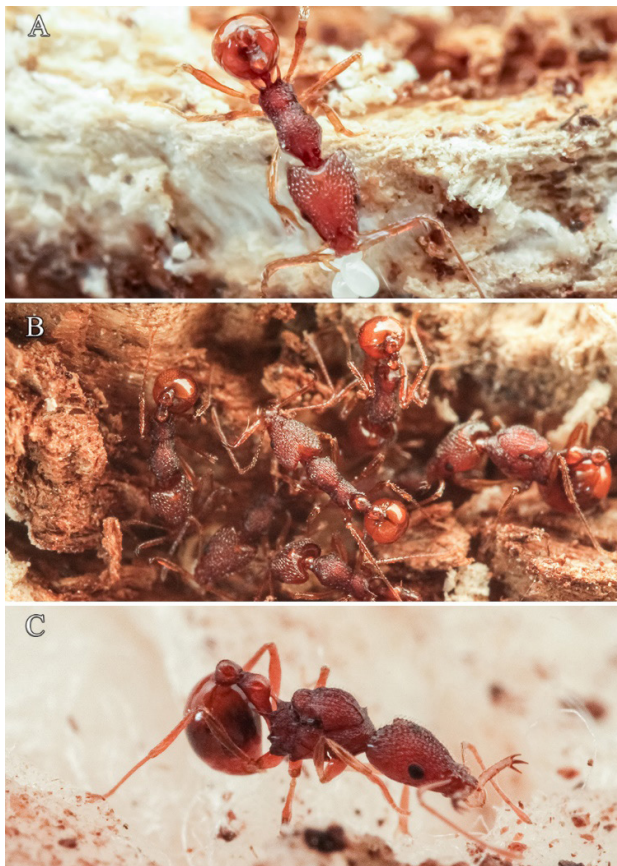
The first images of *A. rudis* larvae are provided (Fig 2). The larval morphology corresponds to the characteristics previously described (Wheeler & Wheeler, 1969) and does not require additional updates. The larvae examined in our study are at a stage comparable to the “very young larva” of its description, as evidenced by the presence of its *meconium*. Notably, *A. rudis* larvae exhibit three distinct types of hair-like projections, as detailed by Wheeler and Wheeler (1969): (i) smaller, bifid hairs, located on the ventral and dorsal regions of all body segments, (ii) medium-sized, slightly straight hairs with a denticulate distal half, mainly present on the ventral region of abdominal segments and on both the ventral and dorsal regions of the head and thorax, and (iii) larger hairs with recurved small anchor-shaped tips, observed on the dorsum of abdominal segments II-V. The anchor-shaped

tips hairs (zoomed in Fig 2a) are a common characteristic among other Myrmicinae species. It has been suggested to play a specialized role in organizing larval spatial distribution into colonies and assisting adult workers in brood care by anchoring the larvae to colony walls (Penick et al., 2012).

*Acanthognathus rudis* adult workers exhibit a unique way of transporting immature individuals using their basal mandibular processes, a behavior previously reported by Brown and Kempf (1969) and imaged for the first time in our study (Fig 3a). Additionally, Brown and Kempf noted that the workers often maintain their mandibles open at 180° angle while inside the nest (Fig 3b). Although we did not observe this specific behavior, our observations revealed that foraging workers of *A. rudis* hold their mandibles open at an approximate 180° angle when outside the nest, when they are threatened or approaching prey in the leaf litter.



**Fig 2.** Larval morphology of *Acanthognathus rudis*: **A.** Larva in the “very young” stage (Wheeler & Wheeler, 1969), zoom indicating type (iii) hairs (right); **B.** Larval head zoomed in profile; **C.** Larval head zoomed in full face view. Abbreviations: **ant.** antennae; **clp.** clypeus; **lbplp.** labial palp; **md.** mandible; **mx.** maxillae; **mxplp.** maxillary palp.



**Fig 3.** Living colony of *Acanthognathus rudis* kept in the laboratory. **A.** Worker carrying the eggs by its basimandibular process; **B.** Workers maintaining the mandible opened at 180° inside the nest; **C.** Queen with gaster in the most common position.

These findings suggest that the mandibles of *A. rudis* remain open for long periods and close primarily for prey capturing, using an ultrafast catapult movement. Another hypothesis from our observations of colony dynamics is that, due to the extremely long length of the mandibles, the elongated basal process may function as “tiny” mandibles, assisting in various tasks beyond brood transport, as noted by Gronenberg et al. (1998).

We also found, for the first time, that workers fold the ventral face of their gaster towards the ventral region of the petiole and mesosoma end (Fig 3c). However, without further studies, we are unable to propose a specific function for this behavior.

In this study, we present the first karyotype description of *A. rudis*, marking the inaugural report for the genus and contributing both to classical and molecular cytogenetic knowledge of the Neotropical *Daceton* genus-group. Additionally, our behavioral observations provide new insights into mandible function in adult *A. rudis* workers. The novel insights from our examination of a single colony highlight the potential for further discoveries within this group, underscoring the need for more comprehensive investigation of *Acanthognathus* species and *Daceton* genus-group.

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## Authors Contribution

GFJ: Conceptualization, formal analysis, investigation, methodology, project administration, writing-original draft, writing-review and editing.

GAT: Formal analysis, investigation, methodology, project administration, writing-review and editing.

EMAV: Formal analysis, investigation, methodology, project administration, writing-review and editing.

DML: Supervision, writing-review and editing.

JES: Supervision, writing-review and editing.

All authors have read and agreed to the published version of the manuscript.

## Data availability

All data generated or analyzed during this study are included in this article and its supplementary information files. Further inquiries can be directed to the corresponding author. High-resolution images of studied specimens of the manuscript were uploaded to the Antweb platform (available at [www.antweb.org](http://www.antweb.org)).

## Competing interests

The authors have no competing interests to declare.

## Statement of Ethics

No ethics approval is required (Ethics Committee on the Use of Animals from the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil).

## References

Baroni Urbani, C. & De Andrade, M.L. (1994). First description of fossil Dacetini ants with a critical analysis of the current classification of the tribe (Amber Collection Stuttgart: Hymenoptera, Formicidae. VI: Dacetini). *Stuttgarter Beiträge zur Naturkunde*, 198: 1-65.

Baroni Urbani, C. & De Andrade, M.L. (2007). The ant tribe Dacetini: limits and constituent genera, with descriptions of new species (Hymenoptera, Formicidae). *Annali del Museo civico di storia naturale Giacomo Doria*, 99: 1-191.

Barros, L.A.C., Chaul, J.C.M., Orivel, J. & de Aguiar, H.J.A.C. (2021). Cytogenetics of *Strumigenys louisianae* Roger, 1863 (Formicidae: Myrmicinae) from Northeastern Amazonia shed light on a difficult species complex. *Zoologischer Anzeiger*, 294: 100-105.

Barros, L.A.C., Chaul, J.C.M., Teixeira, G.A., Lod, R.B., Orivel, J. & de Aguiar, H.J.A.C. (2022). First report of the tramp ant *Technomyrmex vitiensis* Mann, 1921 (Formicidae: Dolichoderinae) in Brazil with cytogenetic and sperm structure data and an updated key to Brazilian Dolichoderinae genera. *Zoological Studies*, 60: e29.

Bolton, B. (1998). Monophyly of the dacetone tribe-group and its component tribes (Hymenoptera: Formicidae). *Bulletin of the British Museum (Natural History) Entomology*, 67:65-78.

Bolton, B. (2000). The ant tribe Dacetini. *Memoirs of the American Entomological Society*, 65: 1-1028.

Bolton, B. (2024). An online catalog of the ants of the world. <http://antcat.org>. (accessed January 30 2024).

Branstetter, M.G., Jesovnik, A., Sosa-Calvo, J., Lloyd, M.W., Faircloth, B.C., Brady, S.G. & Schultz, T.R. (2017). Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *Proceedings of the Royal Society B*, 284: 1-10.

Brown, W.L., Jr. & Kempf, W.W. (1969). A revision of the neotropical dacetone ant genus *Acanthognathus* (Hymenoptera: Formicidae). *Psyche*, 76: 87-109.

Cristiano, M.P., Cardoso, D.C. & Fernandes-Salomão, T.M. (2013). Cytogenetic and molecular analyses reveal a divergence between *Acromyrmex striatus* (Roger, 1863) and other congeneric species: Taxonomic implications. *PLoS One*, 8: e59784.

Crozier, R.H. (1968). The chromosomes of three Australian dacetone ant species (Hymenoptera: Formicidae). *Psyche*, 75: 87-90.

Damasceno, M.T.S., G.A. Teixeira, P.C. Ferreira, R.B. Lod, L.A.C. Barros & H.J.A.C. de Aguiar. (2024). Physical chromosomal mapping of major ribosomal genes in 15 ant species with a review of hypotheses regarding evolution of the number and position of NORs in ants. *Comparative Cytogenetics*, 18: 105-122.

Dietz, B.H. & Brandão, C.R.F. (1993). Comportamento de caça e dieta de *Acanthognathus rudis* Brown & Kempf, com comentários sobre a evolução da predação em Dacetini (Hymenoptera, Formicidae, Myrmicinae). *Revista Brasileira de Entomologia*, 37: 683-692.

Evenhuis, N.L. (2024). The insect and spider collections of the world website. <http://hbs.bishopmuseum.org/codens/> (accessed 26 March 2024).

Galvis, J.P. & Fernández, F. (2009). Ants of Colombia X. *Acanthognathus* with the description of a new species (Hymenoptera: Formicidae). *Revista Colombiana de Entomologia*, 35: 245-249.

GIMP, version 2.10.38. The GIMP Development Team. [Computer Software]. Accessed 08 August 2024. <https://www.gimp.org/>

- Gronenberg, W., Brandão, C.R.F., Dietz, B.H. & Just, S. (1998). Pinkel D., Straume T. & Gray J.W. (1986). Cytogenetic Trap-jaws revisited: the mandibular mechanism of the ant analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences of the United States of America*, 83: 2934-2938.
- Imai, H., Taylor, R.W., Crosland, M.W. & Crozier, R.H. (1988). Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *The Japanese Journal of Genetics*, 63: 159-185.
- Imai, H.T., Crozier, R.H., & Taylor, R.W. (1977). Karyotype evolution in Australian ants. *Chromosoma*, 59: 341-393.
- Jacinto, G.F., Teixeira, G.A., Lopes, D.M., Lino-Neto, J., & Serrão, J.E. (2023). Addendum to the redescription of *Eurhopalothrix reichenspergeri* (Santschi, 1923) (Hymenoptera: Formicidae): larval morphology, cytogenetic and sperm morphometry data. *Zootaxa*, 5352: 443-446
- Janicki, J., Narula, N., Ziegler, M., Guénard, B. & Economo, E.P. (2016). Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: The design and implementation of antmaps.org. *Ecological Informatics*, 32: 185-193.
- Kuznetsova, V., Grozeva, S. & Gokhman, V. (2020). Telomere structure in insects: A review. *Journal of Zoological Systematics and Evolutionary Research*, 58: 127-158.
- Levan, A., Fredga, K. & Sandberg, A.A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas*, 52: 201-220.
- Lopes, L. L., Mariano, C. S. F., Delabie, J. H. C. & Silva, J. G. (2022). First cytogenetic study through conventional staining of the ant genus *Blepharidatta* Wheeler, 1915 (Hymenoptera: Formicidae: Attini). *Sociobiology*, 69: e7843.
- Lorite, P. & Palomeque, T. (2010). Karyotype evolution in ants (Hymenoptera: Formicidae), with a review of the known ant chromosome numbers. *Myrmecological News*, 13: 89-102.
- Penick, C.A., Copple, R.N., Mendez, R.A. & Smith, A.A. (2012). The role of anchor-tipped larval hairs in the organization of ant colonies. *PLoS One*, 7: e41595.
- Pereira, J.O.P. (2006). Diversidade genética da abelha sem ferrão *Melipona quinquefasciata* baseada no sequenciamento das regiões ITS1 parcial e 18S do DNA ribossômico nuclear. D.Sc. Tese, Programa de Pós-graduação em Zootecnia. Universidade Federal do Ceará, Fortaleza-CE, Brazil. 141 pp.
- Schneider, C., Rasband, W. & Eliceiri, K. (2012). ImageJ. *Nature Methods*, 9: 671-675.
- Silva, T.S.R. & Brandão, C.R.F. (2014). Further ergatoid gynes records in the ant tribe Dacetini (Formicidae: Myrmicinae). *Neotropical Entomology*, 43: 161-171.
- Teixeira, G.A., Aguiar, H.J.A.C., Petitclerc, F., Orivel, J., Lopes, D.M. & Barros, L.A.C. (2021). Evolutionary insights into the genomic organization of major ribosomal DNA in ant chromosomes. *Insect Molecular Biology*, 30: 340-354.
- Teixeira, G.A., Barros, L.A.C., Silveira, L.I., Orivel, J., Lopes, D.M. & Aguiar, H.J.A.C. (2022). Karyotype conservation and genomic organization of repetitive sequences in the leaf-cutting ant *Atta cephalotes* (Linnaeus, 1758) (Formicidae: Myrmicinae). *Genome*, 65: 525-535.
- Ulysséa, M.A., Farder-Gomes, C.F. & Prado, L.P. (2024). Biological notes, nest architecture, and morphology of the remarkable ant *Hylomyrma primavesi* Ulysséa, 2021 (Hymenoptera: Formicidae: Myrmicinae). *Myrmecological News*, 34: 1-20.
- Vítková, M., Král, J., Traut, W., Zrzavý, J. & Marec, F. (2005). The evolutionary origin of insect telomeric repeats, (TTAGG)<sub>n</sub>. *Chromosome Research*, 13: 145-156.
- Ward, P.S., Brady, S.G., Fisher, B.L., & Schultz, T.R. (2015). The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, 40: 61-81.
- Wheeler, G.C. & Wheeler, J. (1969). The larva of *Acanthognathus* (Hymenoptera: Formicidae). *Psyche*, 76: 110-113.
- Zerene Stacker, version 1.04. Zerene Systems LLC. [Computer Software]. Accessed 08 August 2024. <https://zerenesystems.com/>

