

The logo for Peer Community In Genomics features a stylized circular network of nodes and lines, with a spiral-like pattern on the left side. The text "Peer Community In Genomics" is displayed in a large, black, serif font to the right of the graphic.

Peer Community In Genomics

A genomic resource for ants, and more

Nadia Ponts based on peer reviews by **Isabel Almudi** and **Nicolas Nègre**

Hugo Darras, Natalia de Souza Araujo, Lyam Baudry, Nadège Guiglielmoni, Pedro Lorite, Martial Marbouty, Fernando Rodriguez, Irina Arkhipova, Romain Koszul, Jean-François Flot, Serge Aron (2022) Chromosome-level genome assembly and annotation of two lineages of the ant *Cataglyphis hispanica*: steppingstones towards genomic studies of hybridogenesis and thermal adaptation in desert ants. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Genomics.

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The ant species *Cataglyphis hispanica* is remarkably well adapted to arid habitats of the Iberian Peninsula where two hybridogenetic lineages co-occur, *i.e.*, queens mating with males from the other lineage produce only non-reproductive hybrid workers whereas reproductive males and females are produced by parthenogenesis (Lavanchy and Schwander, 2019). For these two reasons, the genomes of these lineages, Chis1 and Chis2, are potential gold mines to explore the genetic bases of thermal adaptation and the evolution of alternative reproductive modes.

Nowadays, sequencing technology enables assembling all kinds of genomes provided genomic DNA can be extracted. More difficult to achieve is high-quality assemblies with just as high-quality annotations that are readily available to the community to be used and re-used at will (Byrne et al., 2019; Salzberg, 2019). The challenge was successfully completed by Darras and colleagues, the generated resource being fully available to the community, including scripts and command lines used to obtain the proposed results.

The authors particularly describe that lineage Chis2 has 27 chromosomes, against 26 or 27 for lineage Chis1, with a Robertsonian translocation identified by chromosome conformation capture (Duan et al., 2010, 2012) in the two Queens sequenced. Transcript-supported gene annotation provided 11,290 high-quality gene models. In addition, an ant-tailored annotation pipeline identified 56 different families of repetitive elements in both Chis1 and Chis2 lineages of *C. hispanica* spread in a little over 15 % of the genome. Altogether, the genomes of Chis1 and Chis2 are highly similar and syntenic, with some level of polymorphism raising questions about their evolutionary story timeline. In particular, the uniform distribution of polymorphisms along the genomes shakes up a previous hypothesis of hybridogenetic lineage pairs determined by ancient non-recombining regions (Linksvayer, Busch and Smith, 2013).

I recommend this paper because the science behind is both solid and well-explained. The provided resource is of high quality, and accompanied by a critical exploration of the perspectives brought by the results. These genomes are excellent resources to now go further in exploring the possible events at the genome level that accompanied the remarkable thermal adaptation of the ants *Cataglyphis*, as well as insights into the genetics of hybridogenetic lineages.

Beyond the scientific value of the resources and insights provided by the work performed, I also recommend this article because it is an excellent example of Open Science (Allen and Mehler, 2019; Sarabipour et al., 2019), all data methods and tools being fully and easily accessible to whoever wants/needs it.

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Reviews

Evaluation round #2

Reviewed by **Isabel Almudi**, 31 May 2022

The authors addressed all the comments and concerns rose by the reviewers, thus I recommend it without further review

Reviewed by **Nicolas Nègre**, 22 June 2022

The authors answered to all comments made by the reviewers. I was pleased that a careful evaluation of karyotypes between lineages led to an improved perspective on genome evolution in *Cataglyphis*. All data and pipelines are made available. I therefore recommend acceptance of the manuscript.

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2022.01.07.475286>

Version of the preprint: 1

Authors' reply, 11 May 2022

[Download author's reply](#)

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Decision by **Nadia Ponts**, posted 21 February 2022

Invitation to submit a revised version

Dear authors,

Thank you for submitting to PCI Genomics.

After careful examination of your manuscript and the corresponding external reviews, the value of the paper and the work performed is unanimously recognized. The comments and remarks formulated by the reviewers should nonetheless be addressed prior recommendation. Notably, as noted by both reviewers, discussion putting the findings back in perspective with current biological knowledge and questions is lacking.

As an additional specific comment, considering the level of bioinformatics performed, a schematic drawing of the pipeline describing the sequential analyses performed would be very much welcome as supplemental data for example.

You are kindly invited to submit a revised version of your manuscript addressing all comments and remarks formulated.

Best regards,

Nadia Ponts

Reviewed by **Nicolas Nègre**, 13 February 2022

This manuscript by Hugo Darras et al. presents a high quality genome assembly for two hybridogenic lineages of the ant species *Cataglyphis hispanica*. There are many interest in investigating this model, among which the adaptation to arid condition and more interestingly in my opinion, the unique reproductive mode of hybridogenesis, in which the workers are hybrids between different lineages but the reproductive males and females, are produced by parthenogenesis, thus keeping the lineages genetically separated.

The resources provided in this manuscript are intended to be a first step in using this model in the future and do not pretend to elucidate these questions immediately. Using several genomic technologies including

Illumina, Nanopore and 3C, the authors provide two excellent chromosome scale assemblies for two sampled lineages in Spain: chis1 and chis2 and found that one lineage has 26 chromosomes and the other 27, probably due to Robertsonian translocation. The authors also provide a good quality annotation of genes -predictions being reinforced by RNA-Seq libraries- and of repeat elements. These annotations are not analyzed in depth but only intended as a resource presentation for future studies. Finally, the genomic resource is used to estimate a divergence time of around 1M years between the strains.

Overall, this resource is of high quality, is available publicly and is worth publishing. The methods are appropriately described and the writing is clear with no language issues.

My main criticism of the manuscript is that it does not use this resource to provide some answers to the questions described in the introduction section. Indeed, the only scientific result described is about the change in chromosome numbers. But, unfortunately, while the number of chromosomes has been cytologically confirmed for one lineage, it has not been done for the other one. Thus an additional cytological validation of the translocation can not be provided. **If I had only one request to enhance the paper, would be to provide the karyotype for the Chis1 population as well.** From the same biological material, comparative DNA resequencing (to identify CNVs) and RNA-Seq, could have been attempted to detect differences between the chis1 and chis2 lineages that could explain their divergence. Thus, while the manuscript is robust, it is my opinion it could have had much more impact with additional analyses focused on answering a biological question.

Find below some minor comments:

-Fig1A: In the legend the queen is marked with a red arrow, but I can't see the red arrow (even though the queen individual is obvious).

-Fig.1B: the legend is confusing because the introduction talks about 2 lineages but we immediately see three dots. WGS is defined but not 3C-seq or RNA-seq. And at the end I was confused about which sample was used for which purpose and which one was chis1 and/or chis2. Can it be made clearer on this figure so that the reader understands more easily which material was used?

-l. 247: typo on homozygous

Reviewed by **Isabel Almudi**, 15 February 2022

Darras and coauthors report here the chromosome-level genome assembly of two different lineages of the ant *Cataglyphis hispanica*. For this, they sequenced the genome and transcriptomes of several specimens of two lineages of this species, together with 3C-seq assays, using Nanopore and Illumina technologies. The genomes obtained will be a great resource for the community and will allow further studies about social hybridogenesis, reproduction and other adaptations of these insects.

I have some comments that the authors may want to address:

1. I am missing a bit a phylogenetic perspective, given that the authors performed some comparative genomics analyses. It would be nice if they mention the phylogenetic relationships of the ant species they use for their analyses. They only mentioned that *L. niger* is closer to *Cataglyphis* in phylogenetic terms, but there is nothing about the other species used. They could include a phylogenetic tree with these species highlighted and order them accordingly in the different figures. Perhaps, this would help them to draw some hypotheses regarding the presence of repetitive elements in the distinct genomes.

2. Having the karyotype of the Chis1 lineage would reinforce the results of the manuscript. I understood that it was not possible to collect Chis1 males, would it be possible to obtain a karyotype from hybrid workers, just to confirm that they have 26+27 chromosomes?

3. Could authors clarify what do they mean in lane 160: "no protein similarity and no functional information"? Are they using only ant genomes? Did they search outside ants? Functional information as protein domains?

Minor:

Fig 1. Red arrow pointing towards the queen is missing

Fig 2. There is some discrepancy between the main text and figure 2. In the figure it is stated that Chr 1 from Chis1 is split in Chr 5 and Chr 9, whereas in the text (lane 111), it says that Chr. 1 is split in Chr5 and Chr 10.

Lane 139. Authors claim that chromosomes have small sizes, it would be good if they included some measurements and a scale bar in Figure 3.

Lane 178. There is a mention to Table S1, but I think the authors meant Table S2

Figure S4. There is an overlap of the graph legend with the graph

Lane 196. "smaler " instead of "smaller"