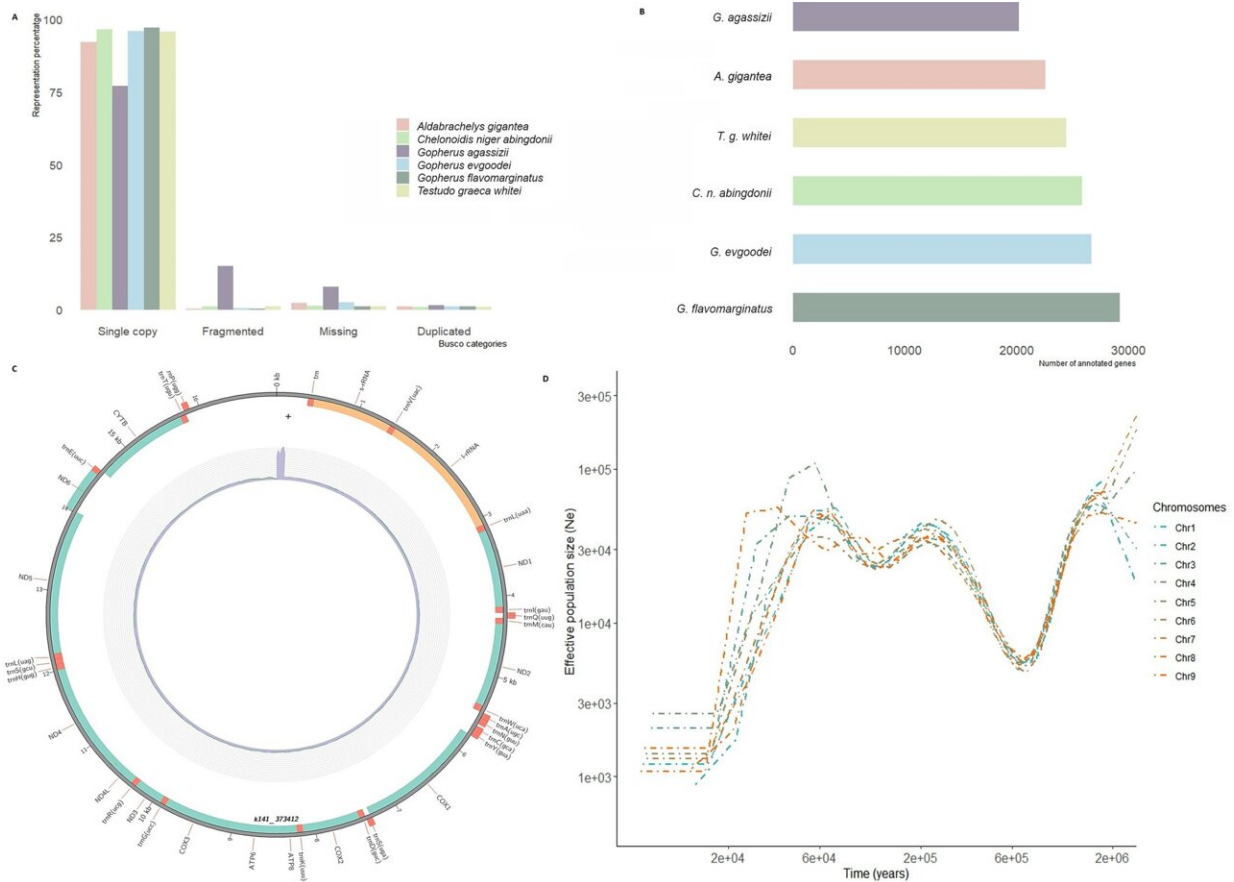


Researchers sequence the genome of the spur-thighed tortoise

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Representation of the main genome assembly results. Credit: *PLOS ONE* (2024). DOI: 10.1371/journal.pone.0303408

Like many threatened land turtle species, the spur-thighed tortoise had

no complete genome. Now for the first time, researchers from the Ecology departments at Miguel Hernández University of Elche (UMH) and the University of Alicante (UA) have successfully sequenced the genome of the spur-thighed tortoise, using the genome of another closely related native American tortoise as a reference.

The results, [published](#) in *PLOS ONE*, will enable the scientific community to support the conservation of these endangered animals.

The spur-thighed tortoise (*Testudo graeca*) is one of the most iconic land tortoise species in the Mediterranean basin. In the Iberian Peninsula, spur-thighed tortoises have two main populations: one in the southeast, from northern Almería to southern Murcia, and another within the Doñana National Park. The species is endangered in Andalusia and is listed in the Catalog of Threatened Species by the Regional Ministry of Murcia and the Ministry of the Environment.

"Understanding the genetic diversity of animals can be very useful for conserving species like the spur-thighed tortoise because the more we know, the better we can understand how these animals have adapted to their environment or what capacity they have to face climate change," explains Andrea Mira Jover, a researcher at UMH and the study's lead author.

In recent years, Mira-Jover adds, [conservation biology](#) has employed a promising tool: [genome](#) sequencing. A genome is the complete set of DNA instructions found in a cell. Sequencing a genome involves reading all the genetic information representative of a species, identifying [specific genes](#), for instance, and organizing them into chromosomes.

The description of this species' genome is a critical scientific milestone, as very few tortoises have been described at this level.

"These results will be a starting point to better understand the evolutionary history of the species and solve questions related to its [life history](#), such as the secret to its longevity," notes UA researcher Roberto Rodríguez-Caro. Moreover, he adds, the publication of this reference genome will provide key tools for its global conservation, as the species is listed as vulnerable by the International Union for Conservation of Nature (IUCN) and requires concrete measures to preserve its populations in the future.

Rodríguez-Caro, from the UA's Department of Ecology, has been studying this species for 15 years, gathering information on its ecology, conservation, and genetics in collaboration with various national and international research centers.

Different techniques exist to obtain complete genomes, depending on whether the genetic information is read in long or short fragments.

"If all the DNA were a novel, some techniques would read long sentences, while others would identify individual words," explains Mira-Jover. Long-read techniques are more effective for assembling *de novo* genomes—organizing DNA sequences without using a previous reference—but they are still too expensive. However, other methods can obtain complete genomes using short-read techniques by referencing the genomes of closely related species.

"In this case, the novel is written using individual words instead of long sentences," the UMH researcher clarifies.

This method, known as "reference assembly," is particularly useful in species with slow evolution, meaning their rate of genetic change is low. They retain the same gene order, called "highly syntenic groups." Using the novel metaphor, if only individual words are available to write a species' genetics, sentences from a similar book can be consulted to

complete the genome.

"An example of slowly evolving organisms is turtles, scientifically known as chelonians or testudines," explains UMH researcher Eva Graciá, study leader and president of the Spanish Herpetological Association. "Chelonians are an ancient and diverse taxonomic group, including freshwater, marine, and land turtles, but their genomic organization is very similar," she points out, adding that "turtles have evolved very slowly throughout history, and their genes are similar and located in the same position on chromosomes."

Land tortoises (Testudinidae) form the most threatened family. Yet, only five reference genomes are available compared to 33 for marine and freshwater turtles. Faced with this situation, the scientific community needs more resources to help conserve land tortoise populations.

For this reason, researchers from the UMH's Ecology department have generated the first chromosome-level reference genome for the spur-thighed tortoise using short-read sequencing techniques. They utilized the known genome of *Gopherus evgoodei*, the Sinaloan thorn scrub tortoise, native to the United States and Mexico deserts.

If the DNA double helix is imagined as a spiral staircase, each step of the staircase would be formed by the so-called "base pairs" containing smaller molecules. The size of a complete genome is measured by the number of base pairs. For example, the human genome has 3.2 billion base pairs containing about 25,000 genes.

Using various bioinformatic techniques, the researchers analyzed a 2.2-billion base pair genome of the spur-thighed tortoise containing nearly 26,000 genes. They also performed a demographic reconstruction to understand the evolutionary history of the [species](#).

"This analysis suggests a very similar evolutionary pattern to what we have already observed with other techniques in previous studies," points out UMH Professor of Ecology Andrés Giménez, author of the publication.

This reference genome will help answer questions about the evolutionary history of the spur-thighed tortoise and for investigating genes of interest in future studies. It will also be a valuable tool for making better conservation decisions. Additionally, the rest of the scientific community will have access to the spur-thighed [tortoise](#) genome, contributing significantly to a field with limited resources.

More information: Andrea Mira-Jover et al, Taking advantage of reference-guided assembly in a slowly-evolving lineage: Application to *Testudo graeca*, *PLOS ONE* (2024). [DOI: 10.1371/journal.pone.0303408](https://doi.org/10.1371/journal.pone.0303408)

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