

Comparative phylogeography of four poorly known Iberian endemic species: *Castellodrilus chitae*, *Ca. ibericus*, *Iberoscolex albolineata* and *Ib. carpetanus*

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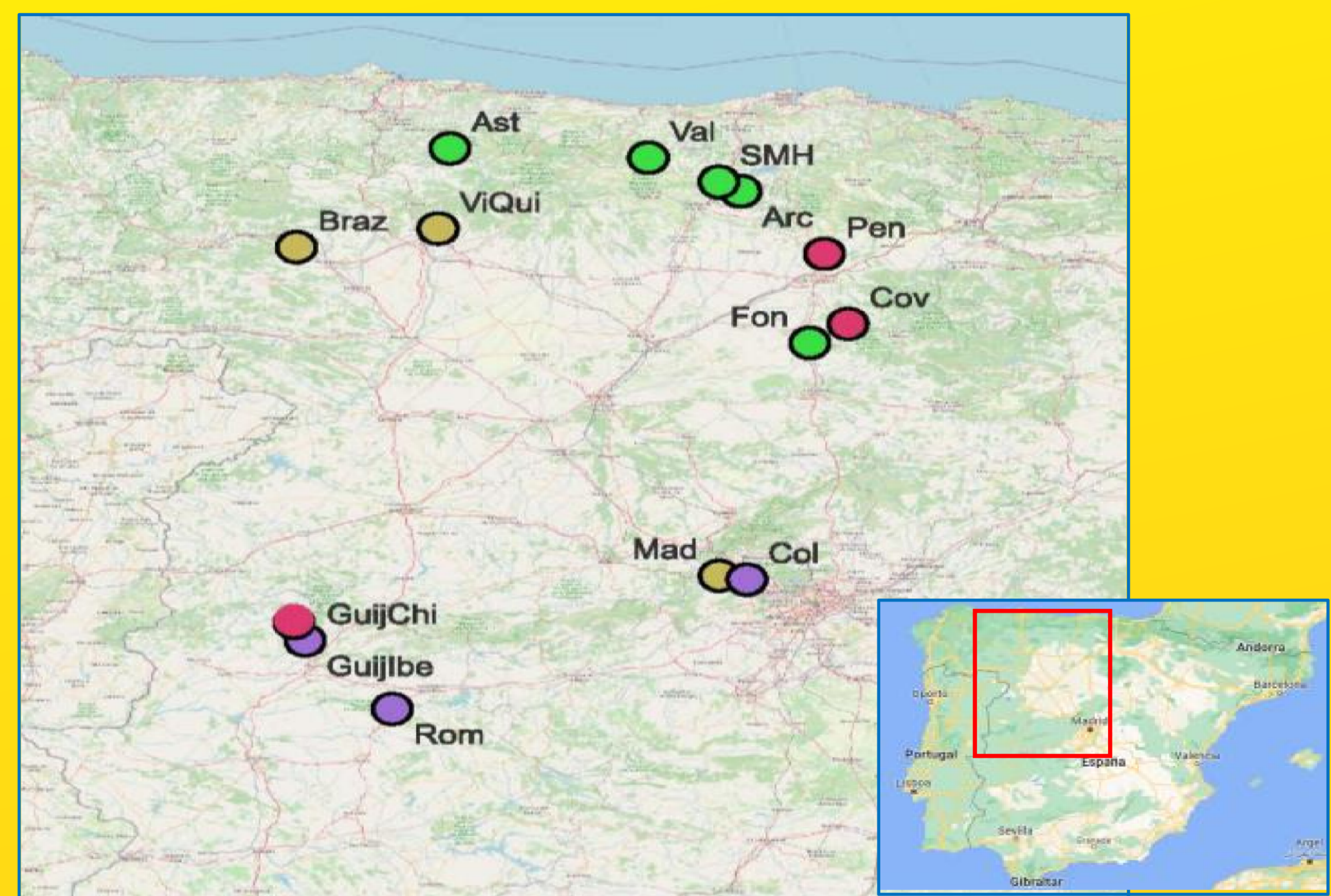
Introduction:

The Iberian Peninsula seems to be a diversification center for earthworm family Lumbricidae as hinted by the presence of several endemisms and early-branching genera that appears only in this area. The Northwestern Iberian Peninsula harbors fewer endemic species than the highly diverse Northeast, but they are characterized by their phylogenetic uniqueness and relatively wide ranges. Comparative phylogeography of representatives of these lineages could provide insight into the influence of geological history biological and ecological differences on their phylogeographic patterns and dispersal ability. Population genetics can also be a powerful tool to assess the diversity of the genetic pool of these endemic species, as a first step to evaluate the conservation status of these poorly known taxa.

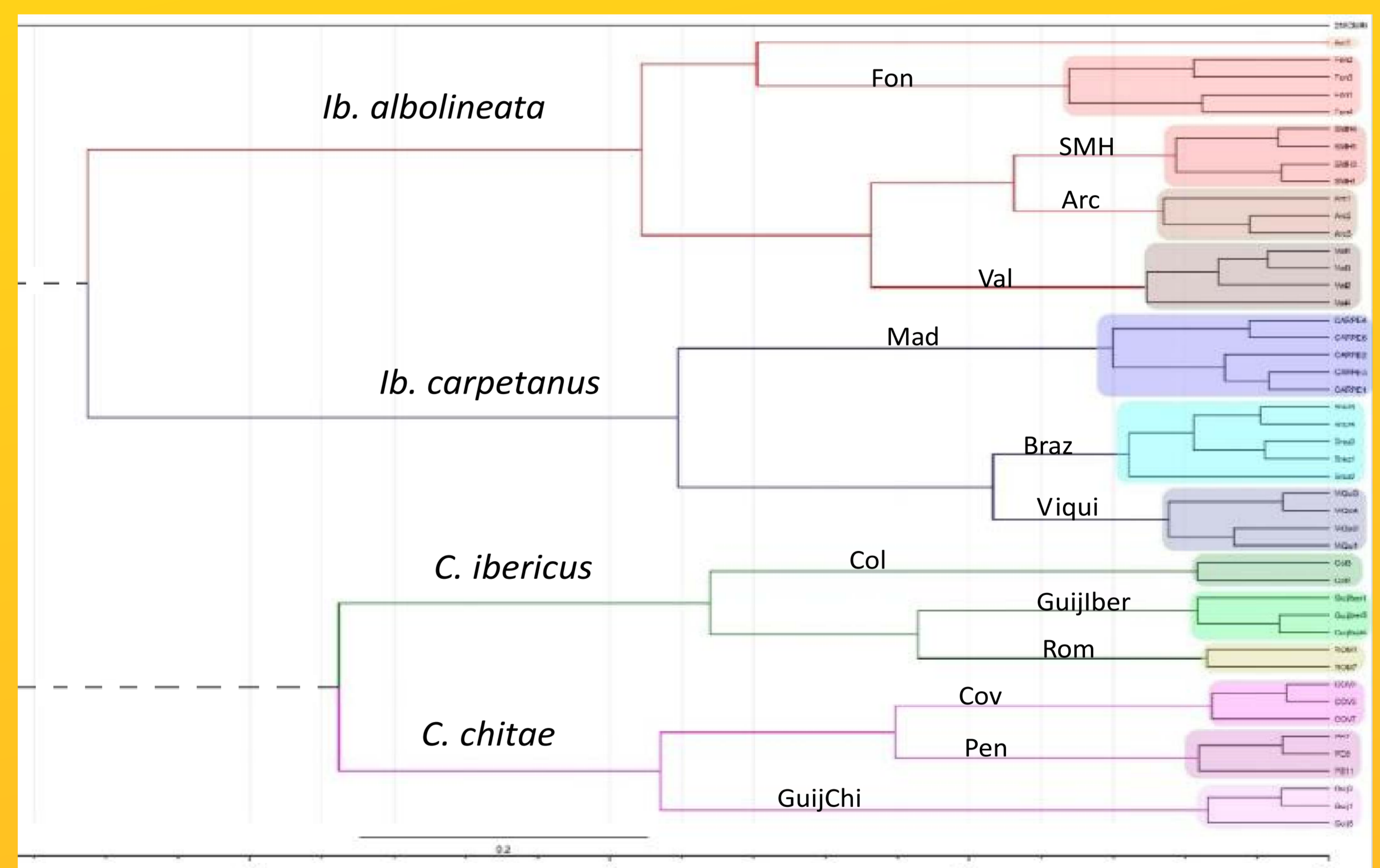
Material and methods:

For this study two species of the early-branching genus *Castellodrilus* (*C. chitae* and *C. ibericus*) and two species of the crown genus *Iberoscolex* (*I. carpetanus* and *I. albolineatus*) were chosen. These species were selected due to their wide, well-known ranges with significant geographical overlap. In total, 13 populations with 5-8 individuals each were studied. Phylogenetic relationships were inferred from the concatenated sequences (molecular markers COI -16S-28S) using a Maximum Likelihood (ML) approach as implemented in RAXML-NG (default parameters). An ultrametric tree was obtained with the software Beast 1.10 (using a relative calibration).

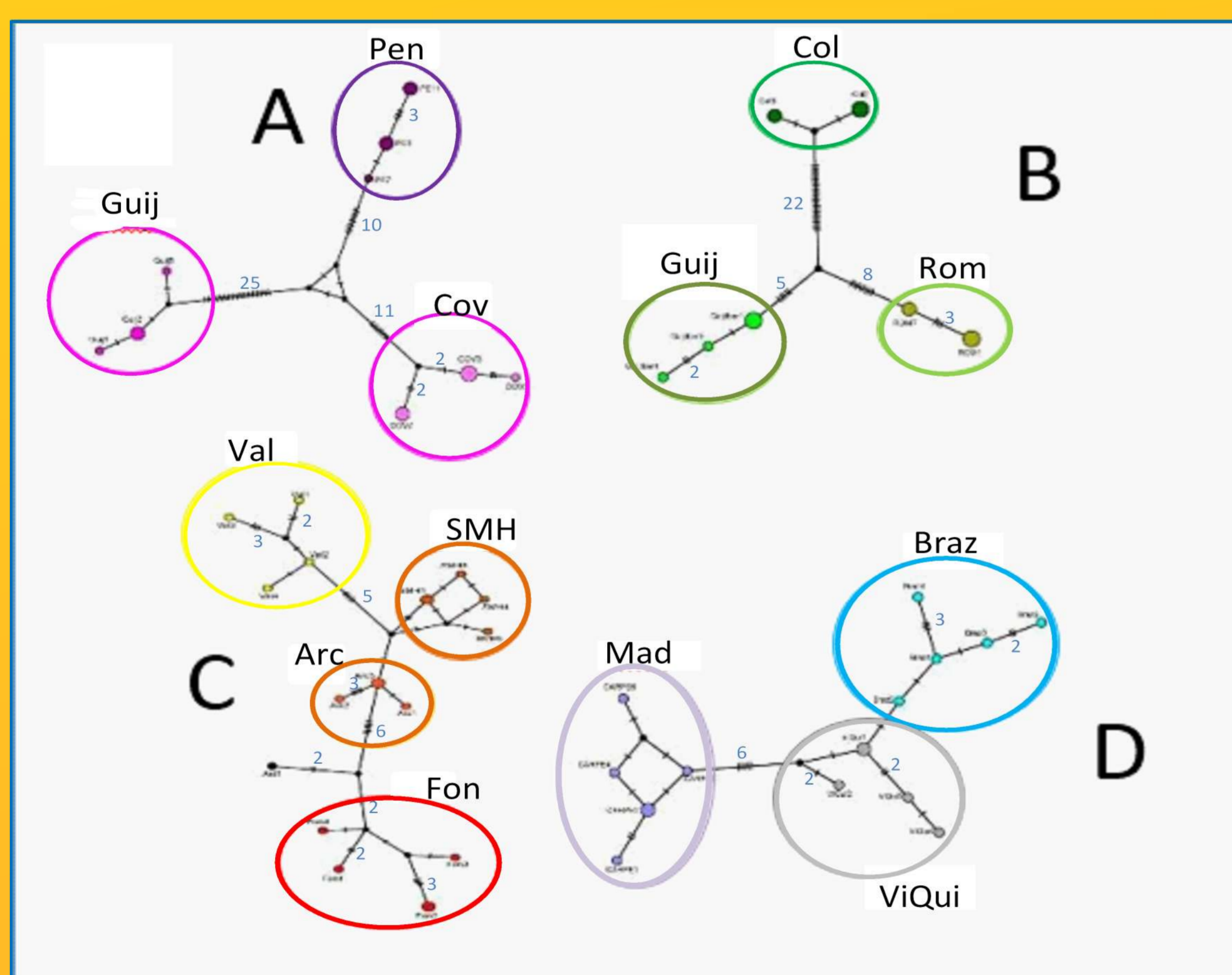
Population genetics parameters were estimated for the COI molecular marker using the software Arlequin 3.1.0.2 and PopArt 1.7.



Map of the populations used in this study Green: *I. albolineata*, Pink: *C. chitae*, Purple: *C. ibericus*, Yellow: *I. carpetanus*. Guij: Guijo de Granadilla, Rom: Romangordo, Fon: Fontioso, Cov: Covarrubias, Pen: Peñahorada, SMH: San Martín de Hoyos, Val: Valdeprado, Arc: Arcera, ViQui: V illaquilambre, Braz: Brazuelo, Mad: El Escorial, Col: Colmenarejo, Ast: Asturias.



Ultrametric tree: Each population is colored differently Populations abbreviations: Same as used in the map.



Haplotype networks of each species: A) *C. chitae*, B) *C. ibericus*, C) *I. albolineatus*, D) *I. carpetanus*. Mutational changes are showed with a number in blue if the changes are greater than 1.

The range of genetic distances for COI between the different populations of each of the species is shown below:

- Ca. ibericus*: 3.2%-5.8%
- Ca. chitae*: 4.8%-7.9%
- I. albolineata*: 1%-2.4%
- I. carpetanus*: 1.2%-2.7%

Results:

*Divergence between the two species of *Castellodrilus* studied is more recent than that of the two species of *Iberoscolex*. Even though *Castellodrilus* has an older origin than *Iberoscolex*, *C. chitae* and *C. ibericus* appear to be more closely related than *I. carpetanus* and *I. albolineatus*.

*Divergence between the populations of each species showed a geographic pattern: less divergent populations were geographically closer. This is also corroborated by the COI genetic distances, which showed a similar pattern. Intraspecific genetic distances in *Castellodrilus* were higher than in the *Iberoscolex*.

The two species of *Iberoscolex* displayed much higher haplotypic diversity ($H = 0.8-1$) and with a greater number of haplotypes per population than the studied populations of *Castellodrilus* ($H = 0.6-0.7$). This suggests that *Iberoscolex* populations could be expanding, with rapid population growth and accumulation of mutations leading to a greater number of haplotypes, while *Castellodrilus* populations appear to be more stable and isolated (with lower gene flow between them).

*The phylogeographic differences between both genera could be related to their ecological categories: *Iberoscolex* are known to be epigeic while *Castellodrilus* is thought to be endogeic (but should be corroborated with modern approaches).

*High genetic diversity suggest that these endemic species can be considered of Low Concern for conservation purposes.