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Solving the puzzle of the colonisation pattern of *Mesoniscus graniger* (Frivaldsky, 1865) in Romania using population genetics

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Mesoniscus Carl, 1906 is a genus of terrestrial isopods (Isopoda: Oniscidea), from the family *Mesoniscidae*, section *Microcheta*. The genus is the only member of the family and contains two cavernicolous species, with a distribution strictly limited to the Alpino-Carpathian chain.

M. alpicola is recorded from the karst regions of the Alps (Italy, Austria, Germany - Bavaria) and lives exclusively at high altitudes, while the more common *M. graniger* has a wider distribution, along the Carpathian Mountains (Slovakia, Poland, Ukraine, Romania, Serbia) and the Dinaric Alps (Serbia, Bosnia and Herzegovina, Croatia, Slovenia). *M. graniger* can also be found in the deeper layers of the soil, not only in caves (Giurginca, 2009). In rare instances, the species *M. graniger* was recorded at low altitudes, on the plains, at a relative proximity to the mountainous areas in which it is usually found (Ferenţi & Covaciu-Marcov, 2018).

The objective of this study is to determine the direction from which the species colonised the Carpathians, using population genetics techniques. In his book, "Aspects concerning the genus Mesoniscus", Giurginca (2009) provided two hypothesis regarding the origin of the genus *Mesoniscus*, described by him as "the Northern Hypothesis" and "the Southern Hypothesis".

Accordong to the Northern Hypothesis the populations would have an Alpine or, perhaps, a Bohemian origin, and they would have migrated southward, through the Carpathians, towards the Dinaric Alps. On the other hand, the Southern Hypothesis suggests the populations would have an Alpine origin, and migrated southwards through the Dinaric Alps and then northward through the Carpathian mountains.

Two independent events - the recurring opening and closing of the seaway from the Mediterranean and the Central Paratethys in Slovenia and the ice cap covering the Bohemian Massif - would block the gene flow between the populations from the Alps and the Carpathians, pushing the two populations towards speciation (Giurginca & Munteanu, 2007).

Based on morphological characters, Giurginca (2009) suggests that *M. alpicola* is a more basal taxon compared to *M. graniger*, so he argues that it is more likely that the origin of the genus is somewhere around the current distribution area of *M. alpicola*: the Alps (Giurginca & Munteanu, 2007).

In Romania, as stated earlier, the only present species from this genus is *M. graniger*. The species has three subspecies identified on the Romanian territory: *M. graniger graniger*, by far the most common subspecies, *M. graniger dragani*, endemic to the Sighiştelului Valley, Bihor Mountains and *M. graniger moldavicus*, present in the Eastern Carpathians (Fig. 1). *M. graniger dragani* has only been found in caves, while *M. graniger moldavicus* has only been found on the surface. *M. graniger graniger* was found both on the surface and underground (Giurginca, 2022).

The populations of *M. graniger* on the Romanian territory are spread following an island model, the populations being limited to the karst regions of the Carpathians, with some exceptions. Fig1 shows where the species has been identified so far in Romania.

A population will be considered a cluster of sites not divided by any natural boundary. In this study, we intend to use the populations with

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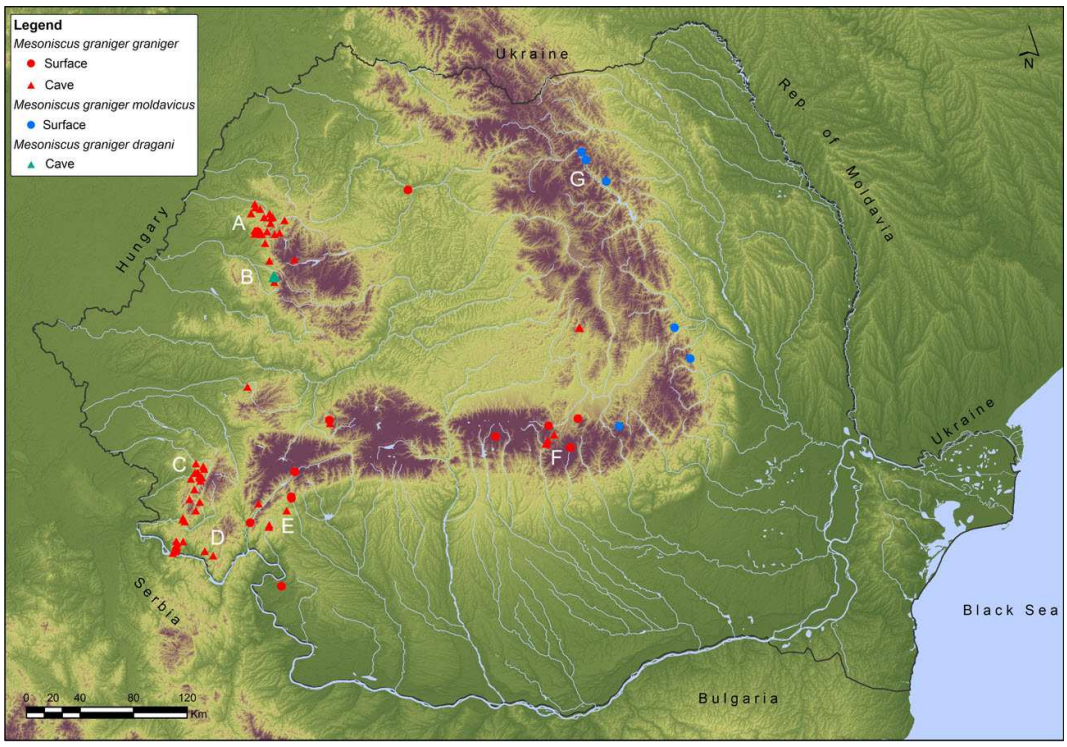


Fig. 1 – Population clusters of *M. graniger* and its subspecies in Romania: A - Apuseni Mountains cluster; B - Bihor Mountains cluster; C - Aninei Mountains cluster; D - Danube Gorge cluster; E - Oltenia cluster; F - Bucegi Mountains cluster; G - Bistriţei Valley cluster.

the largest area and the largest number of individuals, for each subspecies: Population A - *M. graniger graniger* from the Apuseni Mountains, Population B - *M. graniger dragani* from the Bihor Mountains, Sighiştelului Valley, Population C - *M. graniger graniger* from Aninei Mountains (the northern part of Banatului Mountains), Population D - *M. graniger graniger* from the Danube Gorge (the southern part of Banatului Mountains), Population E - *M. graniger graniger* from North-West Oltenia, Population F - *M. graniger graniger* from Bucegi Mountains and Population G - *M. graniger moldavicus* from Bistriţa Valley (Fig. 1).

It is expected that the genetic diversity in the populations furthest from the origin to decrease due to founder effect. The population with the highest genetic diversity is the earliest population to have colonised the Romanian territory and the source population for the others (Schmitt *et al.*, 2002).

The genetic diveristy is measured using the frequence of heterozigosity (*H*), the number of alleles and percentage of polymorphic loci. A population with a high genetic diversity is characterised by a high value of *H*, a high number of alleles for every locus and a high percentage in polymorphic loci (Schmitt *et al.*, 2002). To avoid using loci influenced by natural selection, microsatellites will be used. Ideally, the number of loci chosen and the number of individuals collected are to be as high as possible, for a more accurate description.

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