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EMERGING DISCUSSION

Multiple, solid evidence support that *Austropotamobius italicus* is not native to Spain

Miguel Clavero[1] · **Alejandro Centeno-Cuadros**[2]

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Response to:
Matallanas, B., Ochando, M. D., Alonso, F., & Callejas, C. (2016). Update of genetic information for the white-clawed crayfish in Spain, with new insights into its population genetics and origin. Organisms Diversity & Evolution (in press; doi: 10.1007/s13127-016-0268-4)

Main issues summarised in two sentences:

1- "Taking into account the serious constraints for finding and giving a reliable interpretation of historical documents, genetic evidence should prevail if both lead to different conclusions" (pg 11 last paragraph).
2- [*Austropotamobius italicus*] "seems to be long established in the Iberian Peninsula, at least since the Late Pleistocene, a lapse of time not compatible with a recent introduction by man" (pg 12, first paragraph; similar sentence in abstract).

Main arguments summarised in two sentences:

1- The mono-disciplinary approach followed (and advocated) by Matallanas et al. is much weaker than the transdisciplinary approach used by Clavero et al. (2016), who concluded that there is strong evidence emerging from multiple disciplines indicating that *A. italicus* is not native to Spain.
2- The design of Matallanas et al.'s study is inappropriate to discuss about the native or introduced status of *A. italicus* in Spain, because it included samples from only one Italian population (while sampling 24 Spanish populations). More complete genetic analyses developed in Clavero et al. (2016) do support the non-native status of *A. italicus* in Spain.

On July 14, 2015 a review work on the status of *Austropotamobius* crayfish in Spain co-authored by us (Clavero et al. 2016) was first made available online and B. Matallanas and collaborators first submitted a manuscript to Organisms Diversity & Evolution, which has been recently published (Matallanas *et al.* 2016). Our work had concluded that the knowledge generated by disparate scientific disciplines robustly converged to indicate that *A. italicus* is non-native species in Spain, but Matallanas et al. (2016) argue that *A. italicus* is native to Spain, where it has been present since the Late Pleistocene. Here, we question the conclusions of Matallanas et al. (2016) regarding the status of *A. italicus* in Spain. First, we point out that the mono-disciplinary approach followed by Matallanas et al. (2016) is much weaker than the transdisciplinary approach used and advocated by Clavero et al. (2016). Second, we believe that the genetic patterns presented by Matallanas et al. (2016) are inappropriate to discuss the hypothetical nativeness of Spanish *A. italicus*.

Do not discredit transdisciplinary science

Clavero et al. (2016) collected evidences from several disciplines, including taxonomy, genetics and phylogeography,

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✉ Miguel Clavero
miguelclavero@ebd.csic.es

[1] Estación Biológica de Doñana-CSIC, Americo Vespucio s.n., 41092 Sevilla, Spain

[2] Department of Molecular Biology & Biochemical Engineering, University Pablo de Olavide, Sevilla, Spain

history, linguistics, biogeography, ecology, coevolution and even gastronomy and pharmacy, to show that multiple independent patterns converged robustly to support the non-native status of *A. italicus* in Spain. We provided explicit references to the historical absence of crayfish in Spain, made by Ulisse Aldrovandi, the greatest naturalist of the 16th century worldwide, who had first-hand knowledge of Spain. We transcribed the abundant diplomatic correspondence between Spanish and Tuscan courts negotiating the import of *A. italicus*, which finally took place in 1588. This Tuscan origin of Spanish *A. italicus* is perfectly congruent with the current geographic distribution of the different subspecies of *A. italicus* (e.g., Pedraza-Lara et al. 2010), which is difficultly understandable as a result of purely natural dispersion events. After its introduction, crayfish was mentioned by its Italian name in Spain, since the animal lacked a Spanish name. We highlighted the lack of ectosymbiotic branchiobdellidans in Spanish *A. italicus*, which would be caused by the long-distance transportation from Italy. We also reviewed and analysed mitochondrial DNA (mtDNA) sequences available to date from Spain and Italy, showing that they supported the non-native status of Spanish *A. italicus*.

Clavero et al. (2016) proposed that transdisciplinary approaches surpass single-discipline ones in producing new understanding, a statement that is equally valid for the *A. italicus* case and several others. But Matallanas et al. (2016) seem to suggest that knowledge should be prioritised in terms of some ranking of disciplines: "taking into account the serious constraints for finding and giving a reliable interpretation of historical documents, genetic evidence should prevail if both lead to different conclusions". The problems with this statement are that historical documents are neither scarce nor that difficult to interpret and that constraints are present in all disciplines. Historians could follow the rationale of Matallanas et al. (2016) and argue that given the methodological and analytical problems of associated to genetic studies (e.g., Karl et al. 2012), historical evidences should prevail whenever they are contradicted by genetic results, but this, of course, makes no sense. For the sake of knowledge building, scholars should avoid identifying "winning" and "losing" scientific disciplines and rely more in transdisciplinary learning and collaborations (e.g., Szabó and Hedl 2011). The work of archaeologists, linguists, biogeographers or anthropologists may provide an essential context to interpret patterns in genetic variability. An illustrating example is provided by Seixas et al. (2014), who analysed the history of rabbit (*Oryctolagus cunniculus*) introduction into the island of Mallorca (Spain) based on the variability of the control region of the mtDNA. They found a highly differentiated haplotype group, found exclusively in Mallorca, which had diverged from other haplotype groups up to 170,000 years BP. However, the authors did not propose that rabbit had been present in Majorca since the Middle Pleistocene, because thanks to non-genetic works (e.g., Alcover 2008) they actually *knew* that rabbits could have arrived to Mallorca only after at least 4,300 years BP, when humans first landed in the island. Consequently, Seixas et al. (2014) did not use their genetic results to question the knowledge about the deep history of the Balearic Islands, adapting their interpretation accordingly instead.

Wrong sampling for the question posed

The work by Matallanas et al. (2016) had two main aims: (i) "to study the phylogeographic relationships among Spanish white-clawed crayfish [*A. italicus*] populations" and (ii) "to review the genetic data available regarding their natural or human-mediated origin". The design of the study was accurate for the first aim, but inappropriate for the second. The authors analysed 25 *A. italicus* populations, 24 of which were Spanish, while only one was Italian. This design is not suitable either to obtain conclusions about the relationships of Spanish and Italian populations or to disentangle the real origin of the Spanish populations. If relying on genetic data, the ascertainment of the origin of Spanish *A. italicus* must be unavoidably addressed with a relevant representation of Italian populations. Failing to characterise comprehensively, the genetic variability in putative donor precludes trustable discussion on the status of putatively introduced populations (Bonett et al. 2007). Nonetheless, even well designed genetic studies might be unable to discern the status of Spanish *A. italicus*, because a relevant part of the genetic diversity of Italian populations could have been lost due to the collapse of the species (Clavero et al. 2016). This is why the context setting provided by disciplines other than genetics is critical for interpreting the patterns of genetic variation among *Austropotamobius* crayfish populations.

Matallanas et al. (2016) said that they would "review the genetic data available", which would be a reasonable option to further discuss on the origin of Spanish *A. italicus*. However, they did not perform such a data review. Clavero et al. (2016) did a review and analysis of the whole dataset available of cytochrome *c* oxidase subunit I (COI) sequences of *A. italicus* stored in GenBank and showed that the structure of haplotype networks supported the non-native status of the species in Spain ("Italian" and "Spanish" haplotypes occupied intermixed positions). According to Chapman et al. (2008), the structure of networks is a more reliable tool to assess the status of taxa of unknown origin than the mere existence of private haplotypes, which often occur in non-native populations (e.g., Lejeusne et al. 2014). Matallanas et al. (2016) did not discuss the genetic contribution of Clavero et al. (2016) and used older genetic studies to deny our conclusions regarding the status of *A. italicus*, in spite that those conclusions were built by reviewing the same previous studies.

Matallanas et al. (2016) seem to equal the geographical location of sampled individuals and the long-term geographic belonging of haplotypes. This assumption is wrong, because divergence times inform about the age of the differentiation among haplotypes, not about the place where those haplotypes actually were when the divergence started [see the rabbit example of Seixas et al. (2014)]. Attending to divergence time estimates of up to 14,000 years, Matallanas et al. (2016) concluded that *A. italicus* "seems to be long established in the Iberian Peninsula, at least since the Late Pleistocene". However, divergence times in the range of tens of thousands of years do not seem reasonable between aquatic organisms in Spain and Italy. Populations of several Iberian aquatic organisms have been isolated for much longer periods than those proposed by Matallanas et al. (2016) to mark the divergence between their assumedly "Spanish" and "Italian" crayfish populations (e.g., Gante et al. 2009; Perea et al. 2016). On the other hand, Matallanas et al. (2016) based their estimate of the population expansion up to 14,000 years using mismatch distributions (Rogers and Harpending 1992), which may involve relevant methodological constraints. First, mismatch distributions rely on evolutionary rates (i.e., molecular clocks) that are hardly ever calibrated for the studied species and researchers usually import rates from closely related species (see Baer et al. 2007). Second, Schenekar and Weiss (2011) showed that the mismatch distribution method is frequently applied wrongly (e.g., by confounding divergence rates with mutation rates) and provides estimates of time since expansion within an unknown error range, which arguably varies from <0.05 to tenfold.

Wrapping-up

The multiple evidences provided by Clavero et al. (2016) coincided in signalling a human introduction as the only plausible explanation for the presence of *A. italicus* in Spain. With the available information at hand, any other possibility would be far less parsimonious because several independent facts and patterns should be robustly contradicted to support the native status of the species. This does not mean that we found an incontrovertible truth. Solid evidences could (and should) be used to criticise our conclusions, but they are not found in Matallanas et al. (2016). In summary, pending robust new insights, a human introduction is the only supported hypothesis for the presence of *A. italicus* in Spain.

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