

The white-clawed crayfish in Spain—reply to Clavero and Centeno-Cuadros

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Received: 13 July 2016 / Accepted: 22 July 2016 / Published online: 15 August 2016
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This short communication is only intended to point out briefly and objectively some of the comments, appreciations, and sometimes misinterpretations made by Clavero and Centeno-Cuadros in their criticism to our article on the phylogeography of the white-clawed crayfish in Spain, recently published in this journal.

For those skilled in the discipline of phylogeography, it is well known to be a multidisciplinary science. Therefore, we disagree with the argument of monodisciplinary approach given by these authors.

The work of Matallanas et al. 2016, as all our previous ones, has no intention, no bias, or directionality. It clearly aims what the title synthesizes, an update of genetic information for the white-clawed crayfish in Spain. To achieve this, we have been the first to use two mitochondrial markers, the longest so far used, in a wide and representative sample of Iberian crayfish. A sample of crayfish from Northern Italy was just used as outgroup. The design is, therefore, very suitable and appropriate for the question posed.

Don't discredit transdisciplinary science

We fully agree with Clavero and Centeno-Cuadros on the importance, and also the obvious fact, of integrating evidences from different disciplines for a comprehensive study (i.e., see

This article is a reply to the discussion paper which can be found at <http://dx.doi.org/10.1007/s13127-016-0296-0>.

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Matallanas et al. 2016). We never suggested a knowledge prioritized in terms of some ranking of disciplines, nor “winning” and “losing” scientific disciplines. In many cases, historical documents are scarce and difficult to interpret. As an illustrative example, Clavero and Villero (2014) reported the first written *Austropotamobius italicus* accounts in Spain in 1775 and 1704. Later, Clavero et al. (2015) placed the crayfish arrival in the late sixteenth century by an epistolary document not previously noted. Consequently, the findings of documentary research vary simply by finding an earlier date. The linguistic data and documentary citations they provided as well as their conclusions (Clavero et al. 2015) were not supported at the last Spanish conference on conservation of native crayfish, held on September 2015 (for further details <http://www.lifepotamofauna.org/fitxer/896/ConclusionesjornadasOlot2015v1.pdf>). Likewise, references to crayfish in Spain seem to date back even to name of places from villages of Arab origin as well as to the Spanish popular sayings of that time (Martín-Torrijos et al. 2016, in press, and references therein)

Then, the main problem does not come with transdisciplinary approaches, always welcome, but with the subjectivity in which the analysis of evidences of several of the disciplines mentioned is undertaken. For instance, the analysis of archaeological data by Clavero et al. (2015) is limited to state that “the abundant Iberian archaeological record could be informative for assessing the native status of crayfish. However, there are no reports of crayfish remains from archaeological sites in Spain or Portugal from the last few thousand years”. These authors did not mention at all that in the Italian peninsula, whose archaeological record abundance is out of doubt and where everyone concurs that *Austropotamobius italicus* is also native, the same lack of records happens for the same periods. The same applies (although is also omitted by the authors) to the absence of paleontological findings of *Austropotamobius* in the whole area during the Cenozoic (Garassino pers. com. to Galindo et al. 2014).

Wrong sampling for the question posed

Clavero and Centeno-Cuadros declared that two goals were planned but only one of them was achieved. Notwithstanding in the Introduction we affirmed: The aim of the present work was to study the phylogeographic relationships among Spanish white-clawed crayfish populations and, for conservation purposes, to review the genetic data available regarding their natural or human-mediated origin.

The suitable design of our work, the right and wide sampling for the question posed, the two mitochondrial markers employed—the longest so far—and the powerful set of analyses in this robust data matrix make reliable and accurate the results and conclusions for the aim raised. So the criticism on its design does not make sense. Hence, these solid data allowed us to estimate the time elapsed after the last expansion of this species in Spain, about 25,000 years ago or 14,000 years ago when the two genetic groups detected were considered. It is noticeable to highlight that such time would correspond to what occurred after the last expansion of white-clawed crayfish populations in Spain. Levels of genetic variability and genetic structure of Spanish populations together with demographic inferences suggest that the species is established in the Iberian Peninsula, at least since the Late Pleistocene. Evidently, it must be never understood as the time of divergence between the Spanish and Italian populations.

The presence of genetic structure has been used to argue for the native status of different organisms (i.e., Xavier et al. 2009 cited in Clavero et al. 2015). The substantial distribution patterns of genetic diversity shown at this paper, also by microsatellite markers (Matallanas et al. 2013), seem not compatible with a single introduction of crayfish—or even several—from Italy as late as 1588 onwards. Again, stating that “the weak or absent geographic structure regarding the genetic variability of Spanish crayfish, which is independent from the configuration of river basins (e.g., Pedraza–Lara et al. 2010), clearly suggests a human-mediated distribution pattern” clearly ignores the results shown by our work as well as others (e.g., Diéguez–Uribeondo et al. 2008; Pedraza–Lara et al. 2010) that stress this population structure.

Matallanas et al. (2016) cited the contribution of Clavero et al. (2015) in the work. But their genetic analysis of two networks (COI 5' end and 3' end) from 61 haplotypes unfortunately did not cover the whole dataset available of cytochrome c oxidase subunit I (COI) sequences of *A. italicus* stored in GenBank. The white-clawed crayfish is officially known as *A. pallipes*, and several mitochondrial sequences are deposited in genetic databases under this name, including some of our sequences (i.e., FJ897840–45). Such sequences were not used either. Therefore, these two networks are not complete. Then, we decided not to go in-depth on this particular analysis.

Lastly, it is surprising that “divergence times in the range of tens of thousands of years do not seem

reasonable between aquatic organisms in Spain and Italy” (Clavero and Centeno-Cuadros 2016). Such an extensive statement, taking into account that aquatic freshwater organisms conform a very wide set of living organisms, is backed in only two fish case references. The choice of *Barbus (Luciobarbus) sclateri* by Clavero et al. is unfortunate, as this barbel species belongs to the *Luciobarbus* lineage, whose colonization of Iberia from North Africa is quite well documented (see for example Doadrio et al. 2002). The same reference cited by Clavero et al. provides a very tight schedule to its arrival at Iberia, with a first faunal exchange with Northern Africa occurring at 6.1–6.2 Ma, before the onset of Messinian salinity crisis of the Mediterranean (Garcés et al. 1998; Agustí et al. 2006). A second one occurred between 5.9 and 5.3 Ma, after it and before the opening of the Strait of Gibraltar (Krijgsman et al. 1999; Agustí et al. 2006), geological dated events after which no further interchange of freshwater fauna has taken place. Both, the amount of genetic divergence and the lapse of time associated between *Barbus setivimensis* and *B. sclateri* and within the last species are necessarily shaped by these geological and phylogenetic events. However, none have to do at all with exclusively European genus such as *Austropotamobius* neither its dispersal nor its speciation. Regarding the endemic *Squalius* diversification example, Iberian fish fauna comprises a high percentage of endemic cyprinid species which show this kind of patterns as be expected from the formation of Iberian endorheic (closed) basins. They produced a system of inland lakes in the upper Miocene lakes (11–5 Ma) followed by the formation of river basins (in the Pliocene–Pleistocene, 2.5–1.8) (Filipe et al. 2009). Dispersal and speciation in non-endemic genus or species of fishes and other aquatic taxa present at both sides of the Pyrenees, as is the case of *Austropotamobius italicus*, show quite a different pattern (see for several examples Pedraza–Lara et al. 2010). Divergence times are in the range proposed in our paper or even less (for example, *Barbus meridionalis*, Berrebi et al. 1988) but this is not discussed by Clavero and Centeno-Cuadros (2016).

In short, although in disagreement with most of their arguments, we greatly welcome the feedback from Clavero and Centeno-Cuadros to our recent contribution about the genetic update of the crayfish in Spain. A serious, rigorous, and objective debate certainly enrich the knowledge of this species and will be highly useful for the authorities to be aware of the need to preserve it.

Acknowledgments This work was funded by the Project MCYT CGL2005-05727/BOS and Convenio MMA-UCM 415–2634. During the present work, B. Matallanas received partial funding from the Convenio MMA-UCM.

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