

1 **Multiple, solid evidence support that *Austropotamobius italicus* is not** 2 **native to Spain**

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

21 *Don't discredit transdisciplinary science*

22 Clavero *et al.* (2016) collected evidences from several disciplines, including taxonomy, genetics
23 and phylogeography, history, linguistics, biogeography, ecology, coevolution and even
24 gastronomy and pharmacy, to show that multiple independent patterns converged robustly to
25 support the non-native status of *A. italicus* in Spain. We provided explicit references to the
26 historical absence of crayfish in Spain, made by Ulisse Aldrovandi, the greatest naturalist of the
27 16th century worldwide, who had first-hand knowledge of Spain. We transcribed the abundant
28 diplomatic correspondence between Spanish and Tuscan courts negotiating the import of *A.*
29 *italicus*, which finally took place in 1588. This Tuscan origin of Spanish *A. italicus* is perfectly
30 congruent with the current geographic distribution of the different subspecies of *A. italicus* (e.g.,
31 Pedraza-Lara *et al.*, 2010), which is difficultly understandable as a result of purely natural
32 dispersion events. After its introduction crayfish was mentioned by its Italian name in Spain,
33 since the animal lacked a Spanish name. We highlighted the lack of ecto-symbiotic
34 branchiobdellidans in Spanish *A. italicus*, which would be caused by the long-distance
35 transportation from Italy. We also reviewed and analysed mtDNA sequences available to date
36 from Spain and Italy, showing that they supported the non-native status of Spanish *A. italicus*.

37 Clavero *et al.* (2016) proposed that transdisciplinary approaches surpass single-discipline ones
38 in producing new understanding, a statement that is equally valid for the *A. italicus* case and
39 several others. But Matallanas *et al.* (2016) seem to suggest that knowledge should be
40 prioritized in terms of some ranking of disciplines: "taking into account the serious constraints for
41 finding and giving a reliable interpretation of historical documents, genetic evidence should
42 prevail if both lead to different conclusions". The problems with this statement are that historical
43 documents are neither scarce nor that difficult to interpret and that constraints are present in all
44 disciplines. Historians could follow the rationale of Matallanas *et al.* (2016) and argue that given
45 the methodological and analytical problems of associated to genetic studies (e.g., Karl *et al.*,
46 2012), historical evidences should prevail whenever they are contradicted by genetic results,
47 but this, of course, makes no sense. For the sake of knowledge building, scholars should avoid

48 identifying “winning” and “losing” scientific disciplines and rely more in transdisciplinary learning
49 and collaborations (e.g., Szabó & Hedl, 2011). The work of archaeologists, linguists,
50 biogeographers or anthropologists may provide an essential context to interpret patterns in
51 genetic variability. An illustrating example is provided by Seixas *et al.* (2014), who analysed the
52 history of rabbit (*Oryctolagus cuniculus*) introduction into the island of Mallorca (Spain) based
53 on the variability of the control region of the mtDNA. They found a highly differentiated
54 haplotype group, found exclusively in Mallorca, which had diverged from other up to 170,000
55 years BP. However, the authors did not propose that rabbit had been present in Majorca since
56 the Middle Pleistocene, because thanks to non-genetic works (e.g. Alcover, 2008) they actually
57 knew that rabbits could have arrived to Mallorca only after at least 4,300 years BP, when
58 humans first landed in the island. Consequently, Seixas *et al.* (2014) did not use their genetic
59 results to question the knowledge about the deep history of the Balearic Islands, adapting their
60 interpretation accordingly instead.

61 *Wrong sampling for the question posed*

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

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

90 Matallanas *et al.* (2016) seem to equal the geographical location of sampled individuals and the
91 long-term geographic belonging of haplotypes. This assumption is wrong, because divergence
92 times inform about the age of the differentiation among haplotypes, not about the place where
93 those haplotypes actually were when the divergence started [see the rabbit example of Seixas
94 *et al.* (2014)]. Attending to divergence time estimates of up to 14,000 years, Matallanas *et al.*
95 (2016) concluded that *A. italicus* “seems to be long established in the Iberian Peninsula, at least
96 since the Late Pleistocene”. However, divergence times in the range of tens of thousands of
97 years do not seem reasonable between aquatic organisms in Spain and Italy. Populations of

98 several Iberian aquatic organisms have been isolated for much longer periods than those
99 proposed by Matallanas *et al.* (2016) to mark the divergence between their assumedly
100 “Spanish” and “Italian” crayfish populations (e.g. Gante *et al.*, 2009; Perea *et al.*, 2016). On the
101 other hand, Matallanas *et al.* (2016) based their estimate of the population expansion up to
102 14,000 years using mismatch distributions (Rogers & Harpending, 1992), which may involve
103 relevant methodological constraints. First, mismatch distributions rely on evolutionary rates (i.e.
104 molecular clocks) that are hardly ever calibrated for the studied species and researchers usually
105 import rates from closely related species (see Baer *et al.* 2007). Second, Schenekar & Weiss
106 (2011) showed that the mismatch distribution method is frequently applied wrongly (e.g. by
107 confounding divergence rates with mutation rates) and provides estimates of time since
108 expansion within an unknown error range, which arguably varies from <0.05 to 10-fold.

109 *Wrapping-up*

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

118 **References**

- 119 Alcover, J. A. (2008). The first Mallorcans: prehistoric colonization in the Western
120 Mediterranean. *Journal of World Prehistory*, 21, 19-84.
- 121 Baer, C. F., Miyamoto, M. M., & Denver, D. R. (2007). Mutation rate variation in multicellular
122 eukaryotes: causes and consequences. *Nature Review Genetics*, 8, 619-631.
- 123 Bonett, R. M., Kozak, K. H., Vieites, D. R., Bare, A., Wooten, J. A., & Trauth, S. E. (2007). The
124 importance of comparative phylogeography in diagnosing introduced species: a lesson from
125 the seal salamander, *Desmognathus monticola*. *BMC Ecology*, 7, 7.
- 126 Chapman, J. W., Blakeslee, A. M., Carlton, J. T., & Bellinger, M. R. (2008). Parsimony dictates
127 a human introduction: on the use of genetic and other data to distinguish between the natural
128 and human-mediated invasion of the European snail *Littorina littorea* in North America.
129 *Biological Invasions*, 10, 131-133.
- 130 Clavero, M., Nores, C., Kubersky-Piredda, S., & Centeno-Cuadros, A. (2016). Interdisciplinarity
131 to reconstruct historical introductions: solving the status of cryptogenic crayfish. *Biological*
132 *Reviews* (in press; doi: 10.1111/brv.12205).
- 133 Gante, H. F., Micael, J., Oliva-Paterna, F. J., Doadrio, I., Dowling, T. E., & Alves, M. J. (2009).
134 Diversification within glacial refugia: tempo and mode of evolution of the polytypic fish
135 *Barbus sclateri*. *Molecular Ecology*, 18, 3240-3255.
- 136 Karl, S. A., Toonen, R. J., Grant, W. S., & Bowen, B. W. (2012). Common misconceptions in
137 molecular ecology: echoes of the modern synthesis. *Molecular Ecology*, 21, 4171-4189.
- 138 Lejeusne, C., Saunier, A., Petit, N., Béguer, M., Otani, M., Carlton, J. T., Rico, C. & Green, A. J.
139 (2014). High genetic diversity and absence of founder effects in a worldwide aquatic invader.
140 *Scientific Reports*, 4, 5808.

- 141 Matallanas, B., Ochando, M. D., Alonso, F., & Callejas, C. (2016). Update of genetic information
142 for the white-clawed crayfish in Spain, with new insights into its population genetics and
143 origin. *Organisms Diversity & Evolution* (in press; doi: 10.1007/s13127-016-0268-4)
- 144 Pedraza-Lara, C., Alda, F., Carranza, S., & Doadrio, I. (2010). Mitochondrial DNA structure of
145 the Iberian populations of the white-clawed crayfish, *Austropotamobius italicus italicus*
146 (Faxon, 1914). *Molecular Phylogenetics and Evolution*, 57, 327-342.
- 147 Perea, S., Cobo-Simon, M., & Doadrio, I. (2016). Cenozoic tectonic and climatic events in
148 southern Iberian Peninsula: implications for the evolutionary history of freshwater fish of the
149 genus *Squalius* (Actinopterygii, Cyprinidae). *Molecular Phylogenetics and Evolution*, 97, 155-
150 169.
- 151 Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of
152 pairwise genetic differences. *Molecular Biology and Evolution*, 9, 552–569.
- 153 Schenekar, T., & Weiss, S. (2011). High rate of calculation errors in mismatch distribution
154 analysis results in numerous false inferences of biological importance. *Heredity*, 107, 511–
155 512.
- 156 Seixas, F. A., Juste, J., Campos, P. F., Carneiro, M., Ferrand, N., Alves, P. C., & Melo-Ferreira,
157 J. (2014). Colonization history of Mallorca Island by the European rabbit, *Oryctolagus*
158 *cuniculus*, and the Iberian hare, *Lepus granatensis* (Lagomorpha: Leporidae). *Biological*
159 *Journal of the Linnean Society*, 111, 748-760.
- 160 Szabó, P. & Hedl, R. (2011). Advancing the integration of history and ecology for conservation.
161 *Conservation Biology*, 25, 680-687.