



Unraveling the true identity of the hermit crab *Diogenes pugilator* (Crustacea: Decapoda: Diogenidae) and study of the associated species complex

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Abstract

Diogenes pugilator (Roux, 1829) is a widely distributed species which has been recorded profusely since its description based on specimens from the French Mediterranean. Its actual theoretical distribution covers all east-Atlantic shores, the Mediterranean and the Black Seas. It is very common in shallow waters from 0 to 40 m, especially in soft bottoms and sand beaches, where it can be easily spot, with a extremely long left chelae being dragged on the substrate. During the past decades, several morphotypes have been described based on notorious morphologic variations of different body parts, leading in some cases to the description of new species that years later have been largely synonymized to the original *D. pugilator* from Roux (1829). In his work, Roux gives a short description of the species along with a plate that, although it describes well the general form of the body of the species, it is not detailed enough to be able to compare certain important characteristics. Unfortunately, the type specimens of the Roux's species seem to have disappeared after his death, so the clarification of the true identity of *D. pugilator* remains unclear.

Materials and Methods

In the framework of a more global study devoted to the objective of reviewing Iberian species of hermit crabs both from the morphological and molecular point of view, a review of several specimens identified as *D. pugilator* has been conducted, leading to the identification of a certain number of well-defined morphotypes, all previously included in the nominal species. Specimens studied have been collected by a variety of methods and come from different sources, including Biological Reference Collections (Colección de Crustáceos Decápodos y Estomatópodos del Centro Oceanográfico de Cádiz del IEO- CCDE-IEOCD, "Colección Biológica de Referencia del Institut de Ciències del Mar"- ICM-CSIC and the personal collections from José Enrique García-Raso from Málaga University, Jose A. Cuesta from ICMAN-CSIC, and Christoph Schubart from the University of Regensburg, Germany), from scuba diving, and from scientific cruises devoted to the evaluation of fisheries stocks (ARSA 2017-2018 and MEDITS 2017-2018). Samples were studied under the stereomicroscope and then photographed and sketched to define the main traits defining each of the morphotypes. Drawings were



then digitalized in Inkscape, with the aid of a digital tablet. Molecular samples were then taken from at least a pair of individuals from each of the species, one male and one female. Sequences of two mitochondrial genes (16S and COI) were obtained as well as others from GenBank that were also included in the comparative. A complete Maximum Likelihood phylogenetic tree was obtained for the available species of the genus *Diogenes* along with other species of the family Diogenidae in order to contextualize the phylogenetic position of the species of *Diogenes* and validate morphological differentiation.

Results and Discussion

Preliminary phylogenetic analysis agrees with the morphological delimitation of the morphotypes, suggesting that *D. pugilator* is in fact a species complex composed of several well-delimited species, and that all previous records of *D. pugilator* should be revised under the light of the new information. The discussion about which of the identified morphotypes correspond to the original from Roux (1829), is still unresolved due to the absence of type specimens. Nevertheless, the revision of the synonymized species preserved in the Museum Nationale d'Histoire Naturelle in Paris, along with an increasing sampling effort oriented to the area where the type specimens were initially caught, aims to serve as a clue to elucidate the true identity of the Roux species. Unraveling the true identity of *D. pugilator* is one of the most urgent tasks within this project, but it also opens a series of new challenges, since as the result of the designation of neotypes, other morphotypes need to be addressed and described in detail, updating the biological and distributional information associated with each of them. Once this task is finished, a complete revision of previous records and synonymies for this species should be also conducted and old names, perhaps, resurrected. Nevertheless, although this is still a work in progress, preliminary results suggest that specimens from Atlantic coast of the Iberian peninsula are not assignable to Roux species, which is more likely to be restricted to the Mediterranean Sea. Notwithstanding, two different morphotypes are also present in Iberian Atlantic waters being one of them associated with northern latitudes and the second with southern distribution. There are still many questions to solve around this problem, but it is important to reach an consensus about the definition of the species, given that the absence of original material, can lead to the perpetuation of the misidentifications making studies where these species are involved unreliable and the evaluation of the *Diogenes spp.* populations status, consistently overestimated. See Figure 1 and Figure 2.

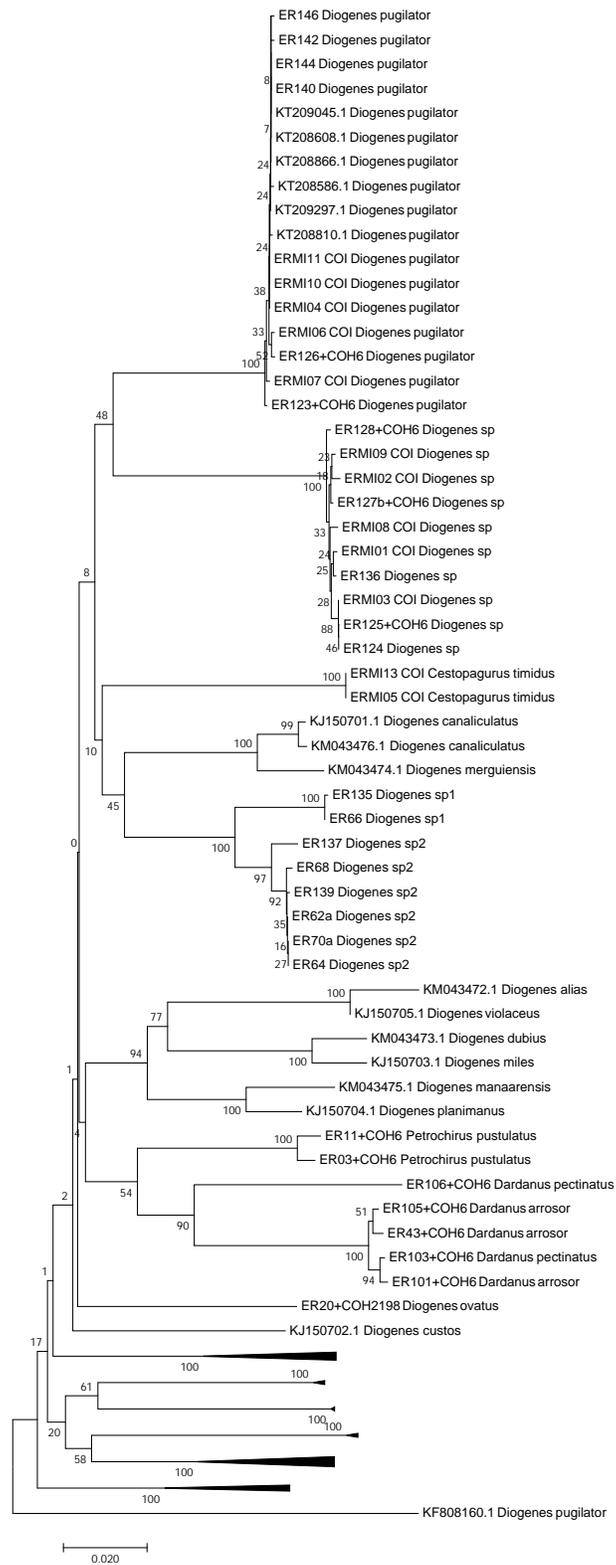


Figure 1: Part of the resulting COI phylogenetic tree showing the different resulting groups involving Diogenes species.



Figure 2: Original illustration from Roux (1829), describing the general morphology of the species.

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