

CRYPTIC DIVERSITY OF THE WIDESPREAD SCORCHED MUSSEL *BRACHIDONTES PHARAONIS-VARIABILIS* SPECIES COMPLEX

Tiziana Curatolo, Matteo Battiata, Sabrina Lo Brutto

Abstract: The phenomenon of bioinvasions, when species spread beyond their native ranges, is widely studied in terms of biological and ecological characteristics of the species involved. However, some cases need taxonomic deepening due to misidentifications which often occur. The *Brachidontes pharaonis-variabilis* species complex, a mussel group, exemplifies these challenges. Originally from the Indo-Pacific region, the species *Brachidontes pharaonis* has successfully invaded the Mediterranean Sea. The true boundaries of its Indo-Pacific range are poorly understood, partly due to confusion with a similar species, *B. variabilis*. This morphological overlap complicates accurate distribution mapping. This paper emphasizes the need for integrative analyses and reports morphometric and genetic variability within the complex.

Keywords: Invasive Alien Species, Mytilidae, Mediterranean Sea, Geometric Morphometry, Genetic analysis

Tiziana Curatolo, DiSTeM, UNIPA, Italy, tiziana3curatolo@gmail.com
Matteo Battiata, University of Palermo, Italy, matteo.battiata@unipa.it, 0009-0003-7164-927X
Sabrina Lo Brutto, University of Palermo, Italy, sabrina.lobrutto@unipa.it, 0000-0002-9964-904X

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Introduction

It is demonstrated that marine biodiversity can be drastically altered by invasive species producing new competitive behaviours or predatory-prey and trophic dynamics [13]. *Brachidontes pharaonis* (P. Fisher, 1870) is one of these; it is one of the most widespread invasive non-indigenous species in the Mediterranean coasts (Figures 1 and 2). This bivalve is a small Lessepsian mussel that has successfully colonised the basin following an immigration pathway through the Suez Canal from the Red Sea [16]. As for many other species, human activities have contributed to *B. pharaonis* dispersion in non-native areas via marine shipping and discharge of ballast water. The presence of this species has direct ecological effects on Mediterranean ecosystems, such as disruption of food chains or displacement of autochthonous mussels [14].

Brachidontes pharaonis is a euryhaline, eurythermal, general filter feeder and presents physiological plasticity which contributed to its expansion [15]. For these reasons, *B. pharaonis* represents a good model for studying the prerequisite conditions for successful colonisation [1, 8, 18, 19]. In the Mediterranean area, it is still in a phase of expansion and its presence is confirmed from east to west in different countries, such as Tunisia, French, Spain, Croatia, Italy, Greece, Turkey, Israel, Egypt, Malta, Slovenia [1, 5, 7, 11, 16]. In particular, the Sicilian Island, in the centre of the basin, seems to be a sort of “trap” for populations which successively disperse by marine currents [3, 16]; the species can there establish local populations that may guarantee the maintenance of the species in the Mediterranean Sea.

The great adaptability and colonisation behaviour are not the only characteristics of *B. pharaonis*. Regarding the Mediterranean area, previous analyses have already shown the co-existence of two mitochondrial haplogroups (M-type and L-type) detected within some sites of the Mediterranean Sea and Red Sea [1, 18, 19]. Moreover, the morphological variation within *B. pharaonis* is so high that the range boundaries are not still defined and are misidentified in regions outside the putative distribution area [3]. Such misidentifications corroborate the difficulties in the taxonomic diagnosis [23]. Several studies in fact have documented the presence of *B. pharaonis* in the Persian Gulf, in Southeast Asia (Indonesia, Malaysia, and Thailand), and in the central Indian Ocean (Sri Lanka) [6, 24, 25]. Hence, we present herein a review of published genetic and morphological data as a supporting baseline for the taxonomic issues of the *Brachidontes pharaonis* + *variabilis* group.



Figure 1 – External and internal view of the valves of two specimens of *Brachidontes pharaonis*.



Figure 2 – Specimens of *Brachidontes pharaonis* in Dahab lagoon, Egypt, Red Sea.

The genetic and morphometric data

The great differentiation detected by molecular markers was the cause of the no longer accepted synonymy of *B. pharaonis* with *B. variabilis* (Krauss), the latter distributed in the Indo-Pacific region [23]. The genetic variation of *B. pharaonis* revealed that the *B. pharaonis* distribution is only limited to the Mediterranean Sea and the northern Red Sea region, as current genetic studies detected [3]. A

Maximum Likelihood tree has been constructed with mitochondrial COI sequences (Figure 3) retrieved from the literature. The phylogenetic tree (Figure 3) shows a great differentiation between sequences from the Red Sea and the Mediterranean area and sequences from the Indian and Pacific Oceans, which cluster in two more separated lineages. A valid example of probable misidentifications are individuals from Sri Lanka which were identified as *B. pharaonis* (Accession Numbers: AJ865780, AJ865781, AJ865786, and AJ865782) but are strongly grouped (99 bootstrap value) in a separate clade together with individuals from China.

The tree of Figure 3 corroborates the existence of three lineages, a finding that is also supported by previous geometric morphometric data of shells [6]. The identification of *B. pharaonis* is complicated. In a case, Swennen et al. [17] used bifurcation of radial ribs, inner margin crenulated on the posterior side, and the colour of the shells for the identification of the species in Thailand while Well et al. [23] suggested a misidentification of samples, which should be identified as *B. variabilis* species.

Consequently, different studies [4, 6, 9] have employed an integrated approach, combining genetic data with geometric morphometrics of valves. This approach aims to reinforce the identification of three lineages within the *Brachidontes pharaonis* + *variabilis* group: those from the Mediterranean and Red Sea, the Indian Ocean, and the Pacific Ocean. In agreement with Curatolo [6] the Geometric Morphometric analysis showed a high shape variation. Here the analysis by Curatolo [6] of the relative warps is reported. The relative warps are principal component vectors of the partial warps, variables generated for thin-plate spline transformations, and were used to describe the major trends in shape variation among specimens [6]. The first relative warp (RW1) explained 45.88 % of the variance, the second (RW3) was 19.78 % and the third relative warp (RW4) 14.98% (the total explained variance was 80.64 %; Figure 4). The thin-plate spline deformation grids shown in Figure 3 (A and B) represented the most extreme shapes in that variation. RW1 included individuals based on the variation in dorsoventral height of the shell, RW2 individuals according to the variation in the ventral margin of the shell (more or lesser concave), while, lastly, RW3 clustered individuals showing the variation in the anterodorsal margin (ranging from angular to curved) (Figure 5).

It is noteworthy that the three lineages align with marine biogeographical provinces [21]. The Red Sea, Western Indian Ocean, and Indo-Polynesian areas are identified as provinces due to their high levels of endemism and isolation from other provinces by barriers [21]. This pattern is also observed in other globally distributed species [2]. The three detected lineages correspond to these provinces: *B. pharaonis*, originating from the Red Sea, has invaded the Mediterranean Sea; *Brachidontes* cf. *variabilis* from Madagascar and South Africa belong to the Western Indian Ocean province; and *B. cf. variabilis* samples from Sri Lanka and China are included in the Indo-Polynesian province.

Records of *Brachidontes pharaonis* or *B. cf. variabilis* from the Indian Ocean and western Pacific regions could belong to three distinct entities which require thorough investigation, and the boundaries of the *B. pharaonis* range need to be clearly delineated, as they are not yet well-defined.



Figure 3 – Maximum Likelihood tree built from COI sequences using published data [6, 12, 17, 19]; a bootstrap value > 0.8 is shown. Location is reported on the name of the tips. The sequences were aligned in BioEdit [10] using ClustaW [20]. The ML analysis was conducted using default parameters for 10000 bootstraps in IQ3 portal [22].

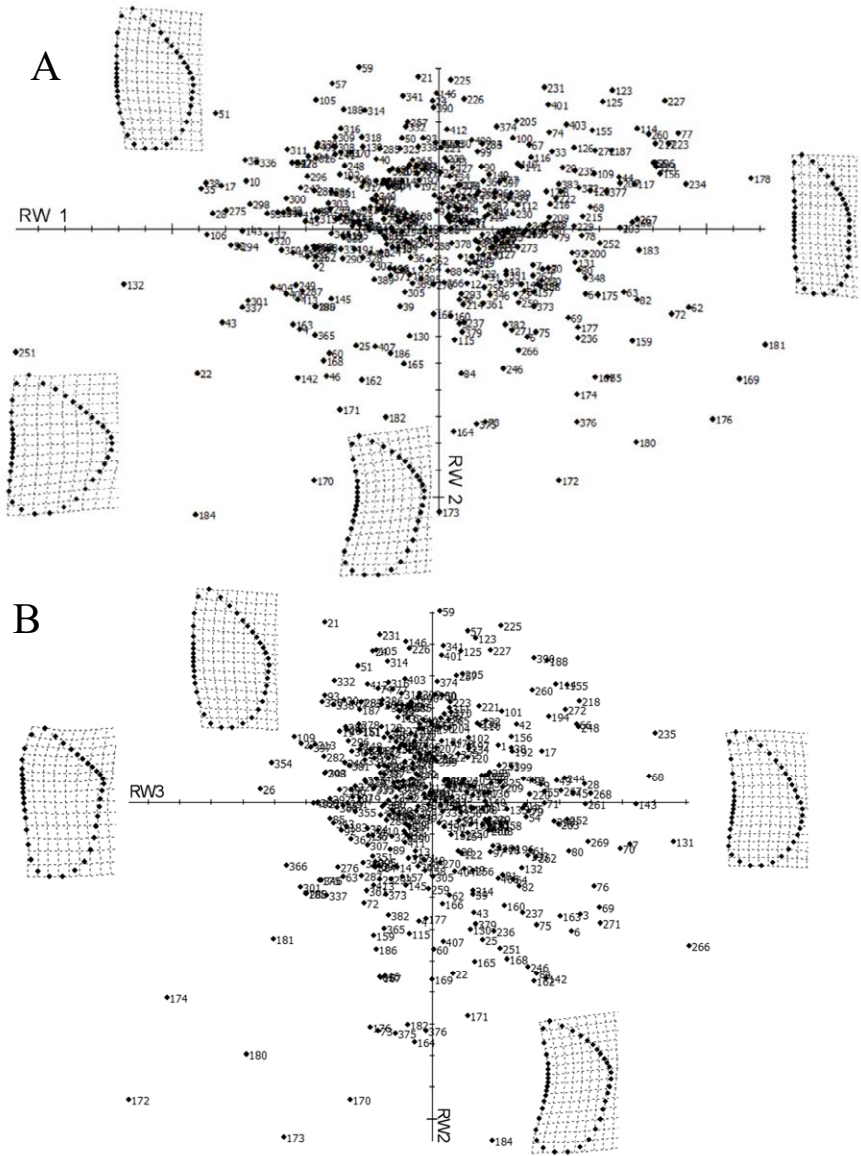


Figure 4 – (A) Scatterplots of the two first relative warp scores obtained from the Relative Warp Analysis (RWA) of the shape of external morphological structures. (B) Scatterplots obtained of the second relative warps scores respect to the third relative warps scores. Plot resulting from the analysis of the shell shape shows deformation grids relative to the axis. (Courtesy Curatolo [6]).



Figure 5 – Illustration of the variable portions of the valve shell in *Brachidontes sp.*

Conclusion

Reports of *B. pharaonis* from regions outside its known range should be treated with caution. At the same time, the *B. variabilis* data from the Indian and Pacific Oceans indicated potential cryptic species. The identification of the natural limits of their maximum range area is one of the most important goals to correctly interpret the presence and dispersion of the species. Additionally, errors in taxonomic identification can undermine good monitoring, creating confusion for the management of the invasive species. This review highlighted the importance of integrative taxonomic approaches to solving various issues about this species complex.

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