

Research Article

On the Fallacy of Color Discrimination: The Rise and Fall of a Rare and Endemic Box Crab (Brachyura: Calappidae)

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Received 27 June 2025; Revised 26 November 2025; Accepted 28 November 2025

Academic Editor: Zhitao Wang

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The Mediterranean Sea is one of the major reservoirs of marine biodiversity and harbors an exceptionally high number of endemic species. Nevertheless, the taxonomic status of many of these endemics still requires confirmation through integrative, modern approaches. We hereby first investigated the identity and status of a rare and endemic box crab, known either as *Calappa rosea* or *Calappa rissoana*, through a multidisciplinary approach that capitalized on the use of nomenclatural rules, museology, integrative taxonomic approaches (morphology, complete mitochondrial genomes, nuclear markers, and phylogenetic analyses), and passive citizen science. Almost all morphological characters failed to differentiate it from the related species *Calappa granulata*, whereas differences in coloration were mostly confirmed here. However, all molecular approaches supported the conspecificity of these two taxa. The combined use of passive citizen science and statistical analyses revealed that the formerly endemic species is an unrecognized ontogenetic stage of *C. granulata*, and, in particular, a rare transitional phase that connects early juveniles to fully developed adults. This renders the investigated taxon a new junior synonym of *C. granulata* and solves nomenclatural and taxonomic ambiguities related to native Mediterranean box crabs, which have remained unsettled for over 200 years. The present study, therefore, provides the first comprehensive reconstruction of the ontogenetic changes occurring in the common Mediterranean box crab throughout its various life stages and raises questions on whether these transitions occur worldwide across box crabs or are restricted to a few species. Finally, it presents new, conspicuous, and detailed morphological and molecular data on the type species of the genus *Calappa*, facilitating future phylogenetic reconstructions and taxonomic assignments within the entire family Calappidae, and discusses the putative occurrence of the other box crabs in the area, suggesting a critical re-evaluation of all historical data and records.

Keywords: *Calappa granulata*; complete mitochondrial genomes; Decapoda; endemic species; integrative taxonomy; ontogenetic changes; passive citizen science

1. Introduction

The north-eastern Atlantic-Mediterranean Sea is a biogeographic region composed of different basins hosting a varied biota that has been the topic of studies since pre-Linnean times [1, 2]. The influx of naturalists and marine biologists exploring the area has already led to the detection

of about 30,000 resident species [3]. However, this number continues to steadily grow due to the ongoing discovery of new species, the previously unnoticed cryptic diversity, and the introduction of non-indigenous species from other biogeographic regions [4–6]. Although most of its resident biota is shared across the different basins, the north-eastern Atlantic-Mediterranean Sea is itself subdivided into two

major bioprovinces: the Atlantic Ocean, which accounts as the donor area of the entire biogeographic region, and the Mediterranean Sea, whose biota largely originated from the Atlantic Ocean following the re-establishment of the Atlantic–Mediterranean connection after the Messinian Salinity Crisis [1, 2, 6]. Despite the continuity between these two basins via the Gibraltar Strait, the significant phylogeographic barriers and the general geographical complexity of the Mediterranean have subsequently contributed to the emergence of endemic, cryptic, and vicariant species that are exclusive to the latter bioprovince, accounting for ~20% of its known taxa [1, 2, 6].

The order Decapoda Latreille, 1802 represents one of the most important invertebrate groups worldwide, and accounts for ~600 recognized species in the region, including “true crabs” (Brachyura), hermit crabs (Anomura), lobsters (Astacidae, Thalassinidea), prawns (Dendrobranchiata), shrimps (Caridea and Stenopodidea), and other lesser-known groups [7]. These species exhibit a remarkable diversity in form, habit, and size due to a wide ecologic and taxonomic/phylogenetic radiation, with extensive speciation events occurring while colonizing every available environment [8]. Approximately half of the European decapod species either co-occur or are exclusively found in the Mediterranean Sea, although data on the local endemism rate are often in disagreement among different authors. For instance, about 40 years ago, Almaça [9] reported that 20%–30% of the known Mediterranean brachyuran species had speciated within the basin. Fifteen years later, d’Udekem d’Acoz [7] estimated that around 50 decapods (~8%) among European taxa were exclusively known from the Mediterranean Sea. More recently, Coll et al. [6] suggested that 10% of the Mediterranean decapod biota was endemic. Indeed, such discrepancies are not only due to different opinions among experts, but also to the marked advance in scientific knowledge, with species once thought to be Mediterranean endemic species that were subsequently found in the nearby Atlantic Ocean [10], or, conversely, with Mediterranean species that were recognized as distinct from their Atlantic counterparts through integrative taxonomic approaches [5]. To further complicate the issue, most Mediterranean endemic species are rather minute and/or sibling taxa, often only known from their type localities or nearby areas and frequently lacking any sort of molecular assessment—for example, *Bresilia corsicana* Forest and Cals, 1977; *Salmonesus sketi* Fransen, 1991; *Anapagurus smythi* Ingle, 1992—for the most updated list see d’Udekem d’Acoz [7]. Expanding their known distribution range and determining whether they are real endemic species or not would require an initial validation through modern methodologies, followed by targeted samplings and specialized taxonomic expertise. Thus, even when confirmed as valid taxa, their true distribution may remain overlooked for decades or even centuries. However, exceptions to this pattern also exist. As an example, the spiny spider crab *Maja squinado* Herbst, 1788, an emblematic megafaunal species that reaches about 25 cm in carapace width, was only recently validated as a Mediterranean endemic taxon, with no intermediates or hybrids ever detected with its sister species *Maja brachydactyla* Balss, 1922

even in the transitional zone between the two bioprovinces [11, 12] and notwithstanding its relatively protracted planktonic larval stage [13], that should theoretically enhance its dispersal potential.

Given all these considerations, and especially in the light of the ever-increasing importance of protecting and discovering biodiversity, it has also become necessary to reevaluate historical and often outdated assumptions using modern approaches. In this study, we definitively cut the Gordian knot on the taxonomic uncertainties surrounding another megafaunal endemic species, namely *Calappa rosea* Jarocki, 1825 (= *Calappa rissoana* Pastore, 1996, junior synonym), through a systematic step-by-step approach. Specifically, we aim to (i) disentangle the nomenclatural issues related to this species and its junior synonyms critically evaluating the literature, applying the rules of the International Code of Zoological Nomenclature (ICZN [14]), and reviewing type specimens and decapod material in relevant institutions; thus, we stabilize nomenclature, taxonomy, and diagnostic features before any further act; (ii) summarize, compare, and reassess the diagnostic features reported by recent authors to distinguish it from the closely related *Calappa granulata* (Linnaeus, 1758), with the final aim to assign the newly collected material to either one or the other taxon; (iii) investigate its status as a rare Mediterranean endemic species using newly collected material and a deep integrative taxonomic approach that incorporates morphology, complete mitochondrial genomes, and phylogenetic analyses carried out on mitochondrial and nuclear markers. After successfully completing these three steps, we further widened our research by (iv) exploring whether the investigated taxon fell within the intraspecific variation or accounts for a distinct color form, anomaly, or ontogenetic stage of *C. granulata* through a data mining and passive citizen science approach coupled with statistical analyses. Therefore, this multidisciplinary study clarifies the taxonomic validity of this species, providing a comprehensive reconstruction of ontogenetic changes occurring from early juveniles to fully developed adults in *C. granulata*. It also presents new, conspicuous, and detailed morphological and molecular data on the type species of the genus *Calappa* Weber, 1795, facilitating future phylogenetic reconstructions and taxonomic assignments within the entire family Calappidae De Haan, 1833.

2. Materials and Methods

2.1. Bibliographic and Museum Research and Nomenclatural Remarks. To ascertain the correct binomial name to be used for the putatively rare and endemic species (*C. rosea* versus *C. rissoana*; see below), but also to stabilize nomenclature and therefore taxonomy and corresponding morphological characters of the native Mediterranean box crabs, a comprehensive review of specialistic literature was conducted. All potential junior synonyms associated with the endemic species were critically evaluated and discussed a priori under the current taxonomic knowledge and the rules established by the International Code of Zoological Nomenclature [14]. This step was flanked by further investigation in museums and additional institutions to potentially trace the type material and, in

general, the specimens related to the historical and recent literature.

Once this preliminary step was achieved, diagnostic characters listed by recent authors dealing with the investigated taxon were screened, summarized in a specific table, and compared with those of *C. granulata* (adult specimens) to identify the samples collected during the present investigation. This was particularly crucial due to the absence of new and accurate taxonomic keys within the family Calappidae worldwide and across the investigated biogeographic area, and due to the wide similarities between box crab species, with characters sometimes partially overlapping between them [15, 16].

2.2. Sample Collection and Morphological Identifications. Newly collected samples were retrieved through the analysis of the bycatch of different marinas, conducted as part of research projects focused on the marine biodiversity of the Mediterranean Sea. Soon after being landed, box crab specimens were placed in cold storage containers and transferred to the Laboratory of Benthos-Naples (SZN, Stazione Zoologica Anton Dohrn, Naples, Italy), where they were promptly washed and brushed to remove mud or pitch and then frozen for the subsequent laboratory steps.

Soon after thawing, sex (male/female) and diagnostic characters resulting from the bibliographic research were assessed visually, with magnifying lenses, or with the use of a Zeiss Axio Zoom.V16 stereomicroscope (Carl Zeiss, Oberkochen, DE). Measurements were taken with a digital Vernier caliper (accuracy ± 0.01 mm) and reported in millimeters. In contrast to some older literature [15], carapace measurements were herein reported as CW $\times$ CL, defined as follows: CW = maximum carapace width including spines and/or lamellae; CL = distance from the tip of the rostrum to the posterior margin of the carapace. Regarding the morphological identifications of the samples, the presence of some characters previously listed as diagnostic, but in effect partially overlapping between the species (see below), led to a reliance on the dominance of sample-specific characters. These characters were divided into (i) unique for *C. rosea*/*C. rissoana*; (ii) unique for *C. granulata*; (iii) shared between the species and/or unassigned.

Once all observations and measurements were obtained, box crabs were transferred to 70%–99.9% ethanol and deposited in the collection of the Laboratory of Benthos-Naples (SZN) with specific voucher codes.

2.3. Molecular Investigation

2.3.1. DNA Extraction and Sequencing. Soon after morphological identifications, total genomic DNAs were extracted from the fifth pereopod of one endemic species and one selected *C. granulata* using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) and following the manufacturer's protocol. DNAs quality and concentration were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

To obtain the seed for the mitochondrial genome assembly, a partial region of the cytochrome c oxidase I (COX1) gene was amplified using the primers LCO1490 5'-GGTCAA

CAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [17]. Polymerase chain reactions (PCRs) were conducted in 25 microliters (μ L) volume reaction, containing 2.5 μ L (10 $\times$) of Roche buffer, 2.5 μ L (2 mM) of Roche dNTPack Mixture, 1 μ L each of forward and reverse primer, 0.25 μ L (5 U/ μ L) of Roche Taq DNA polymerase (all reagents from Sigma-Aldrich, Darmstadt, DE), 1 μ L of template DNA, and sterilized distilled water to 25 μ L. Amplifications were performed as follows: an initial denaturation at 95°C (5 min), followed by 39 cycles of denaturation at 95°C (40 s), annealing at 45°C (40 s), extension at 72°C (40 s), with a final extension at 72°C (5 min). PCR products were visualized by agarose gel electrophoresis (1% agarose), purified using the AMPure XP kit (Beckman Coulter Life Science, Indianapolis, USA), and Sanger sequenced at the SZN Molecular Biology and Sequencing Service through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye Terminator Version 3.1 Cycle Sequencing Kit (Life Technologies, Renfrew, UK). Chromatograms obtained for each sequence were checked, assembled, and edited using Geneious Version 7.1.3, and finally compared with reference sequences from the National Center for Biotechnology Information (NCBI) database using the nucleotide Basic Local Alignment Search Tool (BLASTn, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

To obtain high-throughput sequencing data to assemble mitochondrial genomes and retrieve nuclear genes, the two extracted DNAs mentioned above were shipped to Novogene Company GmbH (Munich, DE) for library construction. A DNA library was constructed for each specimen on the Illumina Novaseq 6000 platform (Illumina, Inc., San Diego, CA, USA) for pair-end (PE) 2 $\times$ 150 base pair (bp) sequencing.

2.3.2. Mitochondrial Genomes Assembly, Annotation, and Comparative Analysis. Raw reads of each library were quality controlled using FastQC-master [18] and cleaned using Trimmomatic [19] with thresholds for minimum Phred scores set to 33 and deleting overrepresented sequences.

Mitochondrial genomes assembly was performed from resulting reads *via de novo* assembly using NOVOPlasty Version 4.2 [20] with default parameters except for: type = mito; genome range = 12,000–22,000; k-mer = 33; read length = 150; insert size = 300; platform = Illumina; Single/Paired = PE. Partial sequences of the COX1 gene previously amplified were used as seed input for the corresponding individual. Mitochondrial elements were annotated using MITOS2 Version 2.1.10 on the Galaxy Europe platform (<https://usegalaxy.eu/>) using the module "Invertebrate". The preliminary resulting annotations were subsequently imported into Geneious for further refinement. Specifically, protein-coding genes (PCGs) were manually inspected to ensure the presence of start and stop codons using Geneious ORF finder and comparing them to those of corresponding genes in other species of the superfamily Calappoidea De Haan, 1833. The boundaries of ribosomal RNAs (rRNAs) were determined by comparing MITOS2 predictions with reference sequences from other Calappoidea taxa. Transfer RNAs (tRNAs) were

identified using tRNAscan-SE Version 2.0 on the above-mentioned Galaxy Europe platform and ARWEN Version 1.2 on the online software (<http://130.235.244.92/ARWEN/>) with default parameters, and finally, if necessary, with manual adjustments.

2.3.3. Phylogenetic Analyses. Due to the paucity of complete mitochondrial genomes of Calappidae—only available for *Calappa bilineata* Ng, Lai and Aungtonya, 2002: see Lu et al. [21]—phylogenetic analyses relied on the same markers used in the most recent studies investigating the family—the mitochondrial COX1 and 16S and the nuclear 28S, H3, and Eno-lase [22]. The two mitochondrial markers were extracted from the previously annotated mitochondrial genomes, while the three nuclear markers were assembled from the reads previously cleaned with Geneious and the Map-to-reference tool (using as reference sequences the barcode regions of interest from phylogenetic-related species), with default parameters except for: sensitivity = custom sensitivity; fine tuning = iterate up to 5 times; minimum overlap identity = 95%. The GenBank nucleotide database was screened using the keywords “*Calappa*” and “COI”, “COX1”, “16S”, “28S”, “H3”, and “Eno-lase”, to find all the sequences of the genus available for the involved genes, together with *Acanthocarpus alexandri* Stimpson, 1871 and *Calappula saussurei* (Rathbun, 1898) to be used as outgroups based on their sister relationships with *Calappa* taxa [22]—last search February 2025. Retrieved sequences were downloaded and imported in Geneious. Moreover, to widen as much as possible the number of taxa or the completeness of the gene dataset, further searches were performed in: (i) the sequence read archive (SRA) database of NCBI, using the keywords “*Calappa* WGS” (Whole Genome Shotgun), and “*Calappa* RNA-seq”. Data were then downloaded using the SRA Toolkit, quality checked using FastQC [18], cleaned using Trimmomatic [19], and selected genes were retrieved using the Map-to-reference tool of Geneious as described above; (ii) The Barcode of Life Data Systems (BOLD) database (<http://www.barcodinglife.org>), using the keyword “*Calappa*”.

Sequences of the five gene fragments were independently aligned using MAFFT Version 7 (<https://mafft.cbrc.jp/alignment/server/>), with default parameters. Ambiguously aligned positions were removed using GBlocks Version 0.91b (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=gblocks&tab_index=1), with the following settings: allow smaller final blocks; allow gap positions within the final blocks; allow less strict flanking positions; do not allow many contiguous nonconserved positions. Finally, single alignments were concatenated using Geneious. The best-fit evolutionary models and partition scheme were selected using ModelFinder [23] implemented in IQ-TREE Version 1.6.8 [24] under the Bayesian information criterion. Bayesian Inference (BI) and maximum likelihood (ML) analyses were conducted. The BI analysis was performed using MrBayes Version 3.2.7 [25] with two independent runs of 10 million generations sampled every 1000 generations, and a standard 25% burn-in. Convergence of the MCMC runs was assessed by reaching an effective

sample size (ESS > 200) for every parameter, and by evaluating posterior distributions using Tracer Version 1.7.1 [26]. The ML analysis was run using IQ-TREE [24] for a total of 1000 replicates and the ultrafast bootstrap feature to assess the robustness of the inferred trees. Obtained trees were visualized in FigTree Version 1.3.1 (<https://tree.bio.ed.ac.uk/software/figtree/>) and edited in Adobe Photoshop CC 2018 Version 19.X.

2.4. Data Mining and Passive Citizen Science From Printed and Web Sources. Color photographs of box crabs (mostly from the Mediterranean Sea, but eventually also from the north-eastern Atlantic) were searched through a systematic screening of printed and web sources (last search: February 2025). These searches were performed manually, as figures appearing in books/field guides and scientific papers are not indexed in databases, and the use of an Application Programming Interface is subject to limitations in some of the web platforms selected [27].

The first phase of data mining focused on data collection. The search for recent (> 1950) books and field guides exploited the 1394 titles housed in the library of the Laboratory of Benthos-Naples (SZN). Printed sources covering decapods and the Mediterranean/European biota were leafed through. In contrast, scientific articles were searched on the Web of Science, Scopus, and Google Scholar databases through a series of queries (“Calappidae”, “*Calappa*”, “*Calappa granulata*”, “*Calappa rosea*”, “*Calappa rissoana*”, “box crab(s)”, “shame-faced crab(s)”, “Mediterranean Sea”), with terms also used in combination among them. Selected articles were also screened for their references. Finally, web sources mostly relied on a passive citizen science approach and capitalized on searches in Google and six social media platforms popular among naturalists and photographers [28], namely Facebook (<https://it-it.facebook.com/>), Flickr (<https://www.flickr.com/>), iNaturalist (<https://www.inaturalist.org/>), Instagram (<https://www.instagram.com/>), Pinterest (<https://it.pinterest.com/>), and X—formerly Twitter (<https://x.com/>). The search was conducted using the previously listed queries along with the species’ common and vernacular names in various languages [29; <https://www.inaturalist.org/taxa/359110-Calappa-granulata—Taxonomy>], with queries categorized as text- (iNaturalist and Google) and hashtag- (Instagram) based, or both (Facebook, Flickr, Pinterest, and X). Preliminary results were manually reviewed, excluding drawings, photos with unnatural colors, species other than *C. granulata*, and any irrelevant content. Encountered photographic records were associated with the corresponding link/printed source in a sheet, with duplicate entries consolidated into single records.

The second phase was devoted to the screening of the records. Individuals were first divided into three different size classes based on literature data—1, small: $CW \leq 7$ cm, corresponding to the maximum size of “*Calappa tuerkayana*” *sensu* Pastore [15] and subsequent literature (references in Innocenti et al. [30]), but also potentially including *C. rosea*/*C. rissoana* and *C. granulata*; 2, medium: $7 < CW \leq 10$ cm, a range only including *C. rosea*/*C. rissoana* and *C. granulata*; 3,

large: CW > 10 cm, a size only reached by *C. granulata*. When sizes were unavailable, the class was inferred from carapace morphology (*C. granulata* develops a larger, smoother, and less convex carapace during growth [15, 30]) and from surrounding elements (e.g., substrata, additional biological entities, human objects). To minimize uncertainties, this step was executed by three different operators (VT, LR, and FC) familiar with the species and the Mediterranean marine environment/biota, with individuals always assigned to the class that received the majority of scores by the three operators. Then, based on the color characters visible in the photos, individuals were divided into two different color classes: (i) “typical”, showing the colors usually reported in adult *C. granulata* as carapace and chelipeds (CC) light orange-yellow-pink with carmine red or dark garnet spots; rostral teeth (RT) orange-pink and generally concolour with CC (both in dorsal and/or ventral view); ambulatory legs (AL) white-light yellow-pink; (ii) “atypical”, showing at least one difference from the coloration pattern reported above. Atypical characters observed in the individuals were finally recorded in the sheet. Noteworthy, to facilitate the subsequent statistical analyses, records illustrating specimens of different sizes or color classes within the same photograph were split into separate entries.

2.5. Statistical Analyses. In the morphological analysis, multidimensional scaling (MDS) plots based on Euclidean distances were used to visualize potential differences between species and among specimens based on seven morphological characters previously listed as diagnostic by past authors (and according to the current bibliographic review), namely: (i) convexity of carapace; (ii–iv) length of rostral teeth, chelipeds, and dactyli; (v) height of crests on propodi; and (vi–vii) shape of sixth abdominal segment and apophysis of antennae.

In the data mining and passive citizen science analysis, both univariate permutational analyses of variance (PERMANOVA [31, 32]) and a rank correlation analysis [33] were used. Data were categorized according to the size classes (1, small; 2, medium; 3, large) and the presence/absence of typical/atypical characters (score range 0–3: 0 = absence of atypical characters; 1 = presence of CC or RT or AL; 2 = presence of CC and RT, or CC and AL, or RT and AL; 3 = presence of CC, RT, and AL). PERMANOVA analyses were conducted to assess the significance of the presence or absence of single and merged atypical and typical characters across the three investigated size classes. Analyses were based on Euclidean distances using untransformed data, with 9999 random permutations of the appropriate units. When significant differences were detected ($p < 0.05$), post-hoc pairwise tests for the fixed factor (size classes) were performed to determine the consistency of these differences. In cases where the number of unique permutations was limited, p -values were obtained using Monte Carlo sampling in the pairwise tests. All analyses were performed using the software PRIMER Version 6, including the PERMANOVA add-on package [34]. The rank correlation analysis was performed to investigate the relationships between the size classes and the score of atypical characters, using the STATISTICA software (Statsoft, France).

3. Results

3.1. Historical Accounts, Nomenclatural Remarks, and Availability and Synonymy of Past and Recent Names. The screening of the taxonomic literature revealed that the investigated species has been potentially listed under three variety names [*Calappa granulata* Var. A. Risso, 1816; *Calappa granulata* Var. Risso, 1826; *Calappa granulata* (Variété) Roux, 1830] and five binomial names [*Calappa rosea* Jarocki, 1825; *Calappa webbii* Risso, 1844; *Calappa webbiana* Monod, 1931 and *Calappa webbiana* Holthuis, 1977 ex Risso manuscript (ms); *Calappa webiana* Monod, 1931 and *Calappa webiana* Holthuis, 1977 ex Risso ms; *Calappa rissoana* Pastore, 1996]. A summary of the main contents of the works involved, as well as of the taxonomic characters listed by the different authors, is reported in chronological order in the Supporting Information 1. Notwithstanding some of these binomial names were often listed as junior synonyms of other taxa and thus were considered as formally valid [16, 35], the majority of them are not available according to the rules established by the International Code of Zoological Nomenclature [14]. This is because they were not published with the use of the binomial nomenclature (Art. 11.4), not followed by a proper description and thus *nomina nuda* (Art. 12, 13, Glossary), or were published as a variety after 1961 (Art. 10.2) or as a junior synonym (Art. 11.6) (discussions under the single binomial names in Supporting Information 1). The sole valid exceptions are represented by two binomial names, namely *C. rosea* Jarocki, 1825 and *C. rissoana* Pastore, 1996.

Among them, *C. rosea* was described by Jarocki [36] with an extremely brief diagnosis and mostly referring to *C. granulata* Var. A. Risso, 1816. However, the fact that Jarocki [36] added nothing to Risso's description suggests that Jarocki never really observed specimens and simply took advantage of the lack of a prior formal description. This hypothesis is also supported by accusations identifying Jarocki as an example of scientific misconduct, including plagiarism and the description of new species merely copying or capitalizing on the work of others [37]. Adding weight to this theory is the absence of any sort of type or box crab material preserved at the Museum and Institute of Zoology of Polish Academy of Science (Warsaw, Poland) (Arkadiusz Cegliński, Dominika Mierzwa-Szymkowiak, and Darek Iwan, personal communications). Therefore, the true identity of *C. rosea* required further investigations, involving an analysis of the three invalid variety names.

The variety of *C. granulata* mentioned/described time by time by Risso [38, 39], Roux [40], and Risso in Holthuis [41] is itself not avoid of taxonomic issues. The few diagnostic characters originally listed by Risso [38] are unstable, as the number of teeth on the posterior and/or on the lateral margins of the carapace of *C. granulata* may vary based on developmental stages, intraspecific variation, and even on the counting method. Additionally, the characteristic coloration of the carapace, pereopods, and nails (pale pink with whitish pereopods and brown nails) falls within the range of intraspecific variability seen in typical *C. granulata* specimens [30].

Moreover, the diagnosis of this putatively single variety was strongly modified over the decades. Roux [40] was the first who made significant strides in describing and illustrating “atypical” box crabs from the Mediterranean Sea, roughly a decade after the variety first appeared in the literature. However, his specimens appear inconsistent with Risso’s original description [38, 39]; thus, it remains uncertain whether Roux truly found and described specimens similar to those initially observed by Risso, or whether he speculated that his findings corresponded to Risso’s previously mentioned variety. To further complicate the matter, it is evident that the description of *C. webbiana* Holthuis, 1977 *ex* Risso *ms* was based solely on bibliographic data without Risso reporting any additional material. In his unpublished manuscript, Risso strongly modified the diagnostic traits he originally listed [38, 39], merely copying Roux’s [40] work. This raises uncertainty as to whether Risso aimed to clarify the identity of his variety or sought to take advantage of Roux’s findings [40] to propose a new species, partially correcting his previous misunderstanding but without drawing much attention to that. Finally, considering the description, shape, and coloration of the specimens figured by Roux [40], it is highly plausible that Roux, at least partially if not completely, dealt with the species later described by Pastore [15] as “*C. tuerkayana*” (now a junior synonym of *C. granulata*: see Innocenti et al. [30]), and that he might even have been the first to hypothesize, or perhaps understand, that the peculiar samples he encountered were or included juveniles of *C. granulata*. Unfortunately, thorough research for the zoological collections of Risso and Roux at the Muséum National d’Histoire Naturelle (Paris, France), Muséum d’Histoire Naturelle de Marseille (Marseille, France), and Muséum d’Histoire Naturelle de Nice (Nice, France) again did not reveal the presence of the box crab specimens mentioned in their papers and manuscripts, which are presumably lost (Laure Corbari, Paula Martin-Lefevre, Vladimir Jecmenica, and Olivier Gerriet, personal communications). Therefore, based on the nomenclatural and historical insights reported here and in Supporting Information 1, as well as on the significant uncertainties that surround this complex issue, it remains challenging to determine whether *C. rosea* can be definitively attributed to one of the two species described later by Pastore [15], or even to typical *C. granulata*. Therefore, the synonymy between *C. rosea* and *C. rissoana* proposed by Holthuis [16] is confuted, and *C. rosea* is provisionally considered here as a *nomen dubium*—ICZN [14]: Glossary.

Calappa rissoana Pastore, 1996, on the contrary, stands out as a more recent taxon with a better-defined diagnosis compared to *C. rosea*. The uncertainties mentioned before about Risso’s and Roux’s varieties were presumably recognized also by Pastore [15], who originally speculated (in the introduction section) that the two specimens from the Gulf of Marseille illustrated by Roux [40] presumably corresponded to both the new species he was describing. However, in the subsequent paragraphs, Pastore first mildly (in the remarks section) and then firmly (in the etymology section) suggested that *C. rissoana* was conspecific with Risso’s unpublished description of *Calappa webbiana* reported by Holthuis [41],

thus again rendering the situation unclear. Unfortunately, the type material designated by Pastore is lost (Giovanni Fanelli personal communication in Innocenti et al. [30]). Nevertheless, thanks also to the inclusion of color photographs in Pastore’s work, as well as to the presence of a proper description, it is highly possible to determine the accurate identity of his species. Consequently, the investigated taxon will be named in the subsequent chapters as *C. rissoana*.

3.2. Diagnostic Characters Based on Literature Data and Identification of the Samples. Although the brief taxonomic key provided by Pastore [15] after the description of the new species only listed just a few characters (mostly based on color features and the number of articles in the antennulae and antennae), the thorough examination of the literature revealed the occurrence of 13 color-based and 17 morphology-based characters published as potentially able to discriminate *C. rissoana* and *C. granulata* (Table 1). The newly sampled material was collected sympatrically from several localities within the Gulfs of Naples and Salerno (Tyrrhenian Sea, central-western Mediterranean Sea), providing valuable validation for the subsequent comparisons. Among the analyzed samples ($n = 22$), one individual (Cal5) was morphologically assigned to the rare *C. rissoana*, whereas the remaining 21 were assigned to the common *C. granulata* (Figure 1; Supporting Information 2 and Supporting Information 3). In general, all color characters previously reported for *C. rissoana* by earlier authors were confirmed in the Cal5 sample, though only seven of these traits proved diagnostic for reliably differentiating the two taxa (Supporting Information 2; Supporting Information 3: Figures S1 and S2). Conversely, with regard to the remaining six characters: (i–ii) two mouthparts-related characters were identical across all samples (Supporting Information 2; Supporting Information 3: Figure S1D, F); (iii–v) a certain degree of variability was noticed in the chelipeds, specifically in the size of the garnet spots and in the color of the posterior edge (Supporting Information 2; Supporting Information 3: Figure S2A,B), but also in the color of the inferior part of the propodus, which was white—a trait shared by both species—in more than half of the samples ($n = 13$) (Supporting Information 2; Supporting Information 3: Figure S2C,D); (vi) the nails appeared very similar among all specimens (Supporting Information 2; Supporting Information 3: Figure S2E,F). On the contrary, the analysis of the morphological characters was more complex. Differences in the carapaces were only partially diagnostic. In the Cal5 specimen, a notably higher convexity of the carapace was found, with a ratio of 0.59 versus 0.48–0.56, as also plotted by MDS (Supporting Information 2; Supporting Information 3: Figure S3A,B; Supporting Information 4). Additionally, the lateral edge was nearly unvarying forward (as described for *C. rissoana*), whereas variations in the profile were observed in all other individuals (Supporting Information 3: Figure S3F,H). Conversely, concerning the other characters: (i) the analysis of the ratio ($L \times 100/W$) used by Pastore [15] for describing and comparing the two species (*C. rissoana*: 78.6–79.2; *C. granulata*: 76.3–77.9) assigned six specimens to *C. rissoana* and four to *C. granulata*, whereas the remaining samples ($n = 12$) did not match any of the two ratios. Notably,

TABLE 1: Differences between *Calappa rissoana* and *Calappa granulata*.

Character	<i>Calappa rissoana</i> <i>fide</i> Pastore [15]	<i>Calappa rissoana</i> (as <i>C. rosea</i>) <i>fide</i> Tiozzo Cuccaro [42]	<i>Calappa granulata</i> (adult specimens)
Color			
Carapace			
General color			
Dorsal side	Bright orange–pink–yellow with 5 rows of garnet spots	—	Light orange–pink with 5 rows of garnet spots
Posterior side	Lighter than dorsal side or gray	—	White–light pink–yellow
Rostral teeth	Often white	White	Orange–pink
Garnet spots	Wider	—	Narrower
Mouthparts			
1 st Maxilliped endopod (endognath)			
Upper part	—	Mottled with red	Mottled with red
External distal part	—	Mottled with red	Light orange–gray
3 rd Maxilliped distal part of exopod (exognath)	—	Bright white	Bright white
Chelipeds			
General color	Bright pink on most areas	—	Light orange–yellow–pink
Posterior edge	Yellow–orange	—	White–pink
Garnet spots	Wider	—	Narrower
Exterior side of propodus (inferior part)	White–pink (brighter)	—	White–light pink–yellow (lighter), often with some yellow tubercles
Pereiopods			
General color	Bright orange–pink–yellow	—	White, light yellow–pink (white merus, yellow propodus)
Nails	(? Light) brown	—	Dark brown
Morphology			
Carapace			
Size range (W × L)	51–80.9 × 42–69.4 (mm)	—	52.4–103.1 × 42–78.1 (mm)
Ratio (L × 100/W)	78.6–79.2	—	76.3–77.9
General shape (convexity)	Longer and less broad, more convex	Less convex	? (Conflicting opinions)
Teeth on posterior edge	More pronounced	—	Less pronounced
Tubercles on posterior region	Single, rarely in series of 2	Smaller in size, smaller number in series of 3	In series of 2 and 3 (mixed)
Rostral teeth (L)	Less pronounced		More pronounced
Lateral edge	Nearly unvarying forward		Varying forward
Mouthparts			
1 st Maxilla outer lacinia (L/W ratio)	Narrower (1.3)	—	Broader
3 rd Maxilliped merus	—	Upper-distal internal part does not exceed upper part of carpus	Upper-distal internal part exceeds upper part of carpus
Chelipeds			
General shape (L)	Shorter	—	Longer
Dactylus (L)	Longer, but more slender	—	Shorter, but less slender
Crests on propodi (H)	Lower	—	Higher
Tubercles on external part of merus	—	More dense, bigger sizes	Less dense, smaller sizes
Abdominal segments			
6 th : General shape (both ♀ and ♂)	Less pronounced, longer and narrower	—	Shorter and wider
Gonopods (pleopods)			
2 nd : General shape	Longer, last article with a narrower curvature	—	Shorter, last article with a wider curvature

TABLE 1: Continued.

Character	<i>Calappa rissoana</i> <i>fide</i> Pastore [15]	<i>Calappa rissoana</i> (as <i>C. rosea</i>) <i>fide</i> Tiozzo Cuccaro [42]	<i>Calappa granulata</i> (adult specimens)
Antennulae (1st Antennae <i>sensu</i> Pastore)			
Number of articles of exterior whip	22–25	—	23–27
Antennae (2nd Antennae <i>sensu</i> Pastore)			
Number of articles	14–16	—	16–17
Apophysis	Lower and wider	—	Higher and narrower

Note: Diagnostic characters of *C. granulata* (adult specimens) rely on those reported by Pastore [15] and Tiozzo Cuccaro [42] (highlighted in bold) and on color features published or inferred from recent papers and samples that report the two species as separate taxa (Noël [29]; Innocenti et al. [30]). Abbreviations: H, height; L, length; W, width.

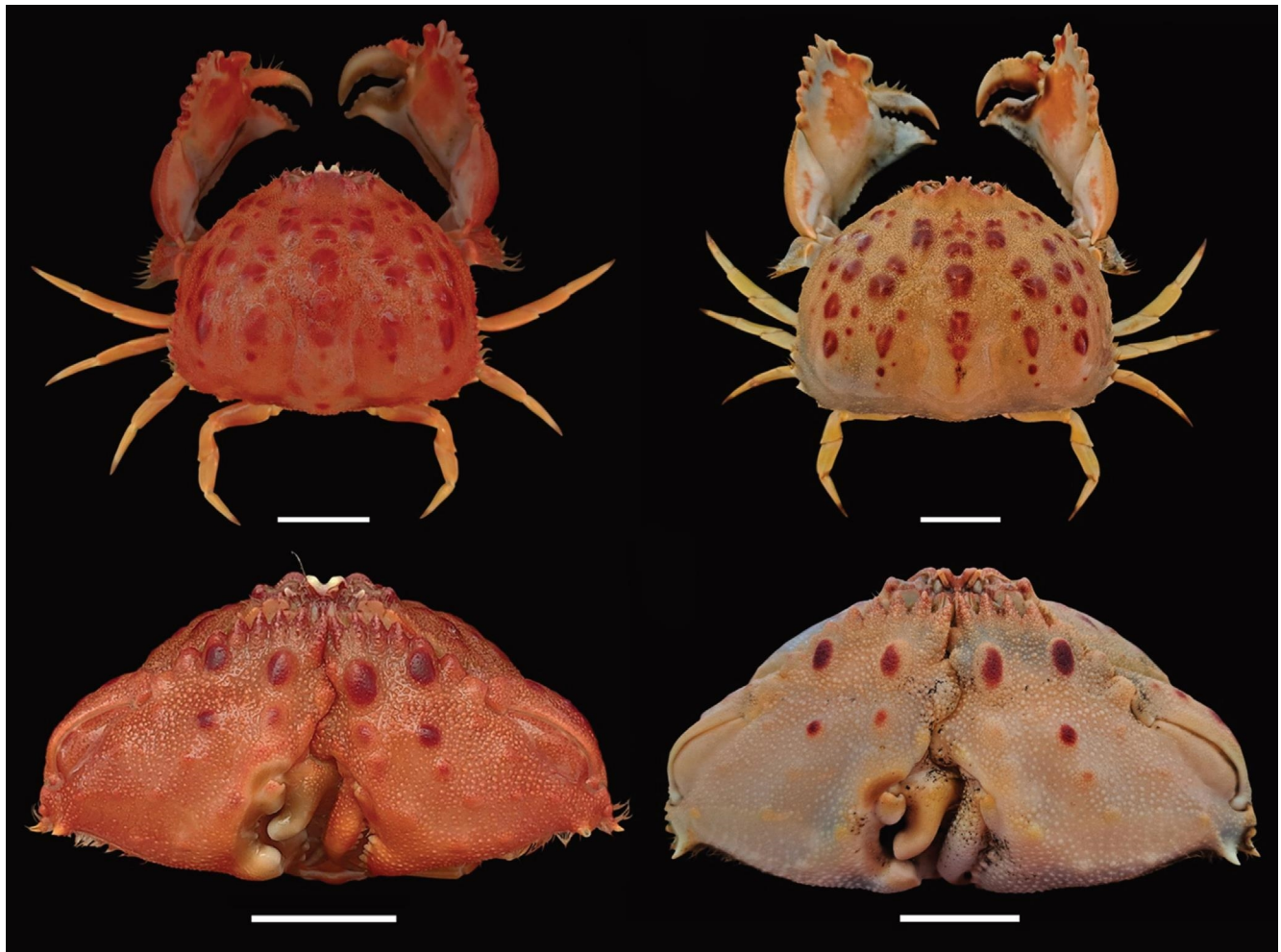


FIGURE 1: Dorsal and frontal views of *Calappa rissoana* (Cal5, voucher SZN_B_3859CR99F, CW × CL: 69.35 × 54.84) and *Calappa granulata* (Cal6, voucher SZN_B_4115CR99H, CW × CL: 96.15 × 73.37). Scale bars: 2 cm.

not even the holotype of *C. rissoana* matches the ratio published for the species (42.6 × 52.0 mm, yielding a ratio of 81.9); (ii) the teeth on the posterior edge were pronounced across all samples (Supporting Information 2; Supporting Information 3: Figure S3A,B); (iii) the tubercles on the posterior region consistently ranged from single ones to series of three, though their dominance varied (Supporting Information 2;

Supporting Information 3: Figure S3C,D); (iv) the length of the rostral teeth exhibited a significant variability, with seven individuals showing a ratio ≥3, but no clear pattern emerged overall (Supporting Information 2; Supporting Information 3: Figure S3E,G; Supporting Information 4). The analysis of the diagnostic mouthpart features ascribed eight specimens to *C. rissoana*—ratio ~1.3 according to Pastore [15]—and 14

to *C. granulata* based on the outer lacinia of the first maxilla (Supporting Information 2; Supporting Information 3: Figure S3J,L), whereas, in all samples, the upper-distal internal part of the merus extended beyond the upper part of carpus in the third maxilliped, as published for *C. rissoana* (Supporting Information 2; Supporting Information 3: Figure S3I,K). Differences in chelipeds were not truly diagnostic. Indeed, although the specimen Cal5 was always among those ranking with smaller measurements, the ratios produced here were similar across all samples, and the same holds true when analyzing the ratios on the length of dactyli and the height of the crests on propodi, although also these may indeed appear shorter in Cal5 (Supporting Information 2; Supporting Information 3: Figure S4A,C; Supporting Information 4). No clear pattern either emerged regarding the tubercles on the external part of merus in the chelipeds, although specimens with larger carapace widths generally displayed sparse and mostly small tubercles, while those with smaller widths apparently exhibited denser, predominantly large tubercles (Supporting Information 2; Supporting Information 3: Figure S4B,D). Variability was also noted in the shape of the sixth abdominal segment, and all males displayed a second gonopod as typical for *C. granulata* (as figured in Innocenti et al. [30]) (Supporting Information 2; Supporting Information 3: Figure S4F,H; Supporting Information 4). Finally, no clear pattern was noticed in the shape of the apophyses of antennae (Supporting Information 2; Supporting Information 3: Figure S4M,O; Supporting Information 4). Surprisingly, mismatches arose even when considering the two morphological characters outlined in Pastore's [15] brief taxonomic key and regarding the articles of (i) the antennulae, with more than half of the specimens ($n = 14$) that exhibited a number of articles within the overlap range between the two species (23–25), whereas two samples matched *C. rissoana*, three matched *C. granulata*, and three had 21 articles, falling outside both published ranges (*C. rissoana*: 22–25; *C. granulata*: 23–27) (Supporting Information 2; Supporting Information 3: Figure S4I–L); (ii) the antennae, with all specimens that showed a number of articles (7–13) below the published ranges (*C. rissoana*: 14–16; *C. granulata*: 16–17) (Supporting Information 2; Supporting Information 3: Figure S4M–P).

To further test the putative differences between the two taxa, the specimens Cal5 (19 characters unique for *C. rissoana*, nine characters shared and/or unassigned, one character unique for *C. granulata*: voucher SZN_B_3859CR99F) and Cal6 (15 characters unique for *C. granulata*, eight characters shared and/or unassigned, six characters unique for *C. rissoana*: voucher SZN_B_4115CR99H), respectively assigned to *C. rissoana* and *C. granulata* and showing the highest dominance within the characters listed in the literature, were selected for the subsequent molecular work.

3.3. Mitochondrial Genomes Organization and Comparison. PCRs amplifications yielded two partial COX1 gene sequences of 608 base pairs (bp), showing among them a similarity value of 99.84% and differing in a single nucleotide (a transition between A and G). BLASTn hits for both species returned high similarities (98.61%–100%) with several sequences—

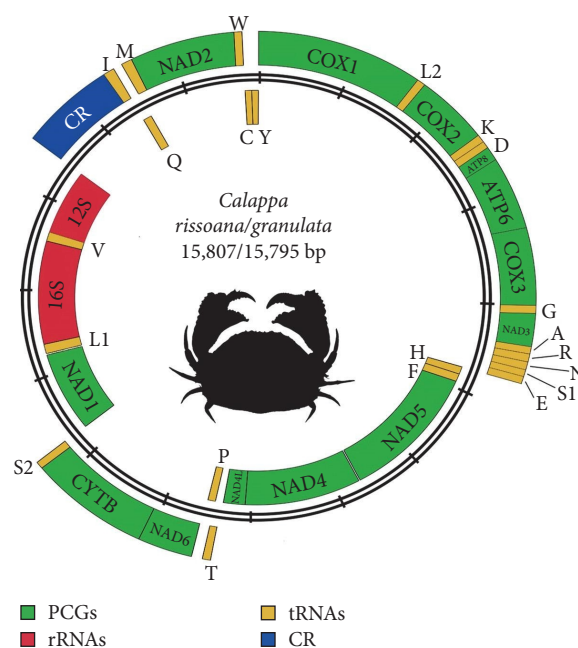


FIGURE 2: Map of the mitochondrial genomes of *Calappa rissoana* (GenBank PV582136) and *Calappa granulata* (GenBank PV567534). The two circles show the different strands (external = heavy (+) strand; internal = light (–) strand). Bars on the circles are placed each 1000 bp. Genes are located based on the encoding direction (outside = heavy strand; inside = light strand). Standard abbreviations follow those reported in the text and in Table 2.

JQ306054, MN107292–294, MW915792–804, and OM653503–513 [30, 43, 44, Vella et al., unpublished]—ascribed to *C. granulata*, thus excluding contaminations.

Mitochondrial genomes resulted in double-stranded circular molecules of 15,807 bp (*C. rissoana*) and 15,795 bp (*C. granulata*), both composed of 13 PCGs, two rRNAs, 22 tRNAs, and a putative control region (CR). Among them, 23 genes were encoded on the heavy (+) strand, while four PCGs, two rRNAs, and eight tRNAs were encoded on the light (–) strand (Figure 2). The total PCGs were 11,194 bp in size (70.82%: *C. rissoana*; 70.87%: *C. granulata*). In both species, all the PCGs had the same length and the same start and stop codons. Amino acid translation proved identical, but 7 differences in nucleotide sequences were noticed. The total rRNAs were 2150 bp in size (13.60%: *C. rissoana*; 13.61%: *C. granulata*) and proved identical in length and nucleotide sequences in both the 12S and the 16S genes. Total tRNAs ranged from 1456 bp (*C. granulata*) to 1459 bp (*C. rissoana*) (9.23%: *C. rissoana*; 9.24%: *C. granulata*), with single tRNAs ranging from 62 to 71 bp. One single nucleotide differed among the species. The CR was the most variable region, ranging from 915 bp (*C. granulata*) to 924 bp (*C. rissoana*) (5.84%: *C. rissoana*; 5.79%: *C. granulata*). Moreover, 11 differences in nucleotide sequences were noticed due to four transversions and seven transitions. The overlapping regions (all of 74 bp) were identical among species and located at 11 gene junctures. Finally, the intergenic regions were 160 bp in size (1.01% of both species), with the longest one (13 bp) located between the NAD1 gene and the tRNA-Leu1. The only difference between

TABLE 2: Mitochondrial genomes organization and comparison between *Calappa rissoana* and *Calappa granulata* (numbers in parentheses).

Gene	Position		SD	Length	Codon		Laa	Ign	Difference
	Start	Stop			Start	Stop			
COX1 ^a	1	1539	+	1539	ATG	TAA	512	−5	456 (G-A)
tRNA-Leu (L2) (tta)	1535	1598	+	64	—	—	—	18	—
COX2 ^a	1617	2321	+	705	ATG	TAA	234	−20	—
tRNA-Lys (K) (ttt)	2302	2371	+	70	—	—	—	0	—
tRNA-Asp (D) (gtc)	2372	2438	+	67	—	—	—	0	—
ATP8 ^a	2439	2597	+	159	ATG	TAG	52	−4	—
ATP6 ^a	2594	3265	+	672	ATA	TAA	223	−1	141 (A-G)
COX3 ^a	3265	4056	+	792	ATG	TAA	263	2	—
tRNA-Gly (G) (tcc)	4059	4121	+	63	—	—	—	−3	—
NAD3 ^a	4119	4475	+	357	ATA	TAA	118	5	—
tRNA-Ala (A) (tgc)	4481	4544	+	64	—	—	—	10	—
tRNA-Arg (R) (tgc)	4555	4616	+	62	—	—	—	0	—
tRNA-Asn (N) (gtt)	4617	4684	+	68	—	—	—	0	—
tRNA-Ser (S1) (tct)	4685	4750	+	66	—	—	—	0	—
tRNA-Glu (E) (ttc)	4751	4815 (−1)	+	65 (−1)	—	—	—	22	—
tRNA-His (H) (gtg)	4902 (−1)	4838 (−1)	−	65	—	—	—	0	—
tRNA-Phe (F) (gaa)	4966 (−1)	4903 (−1)	−	64	—	—	—	3	—
NAD5 ^a	6698 (−1)	4964 (−1)	−	1735	ATG	T	578	21	—
NAD4 ^a	8054 (−1)	6720 (−1)	−	1335	ATG	TAA	444	−7	—
NAD4L ^a	8350 (−1)	8048 (−1)	−	303	ATG	TAA	100	18 ^b	—
tRNA-Thr (T) (tgt)	8369 (−1)	8437 (−3)	+	69 (−2)	—	—	—	0	—
tRNA-Pro (P) (tgg)	8503 (−3)	8438 (−3)	−	66	—	—	—	2	—
NAD6 ^a	8506 (−3)	9012 (−3)	+	507	ATT	TAA	168	−1	—
CytB ^a	9012 (−3)	10,148 (−3)	+	1137	ATG	TAA	378	−1	174 (C-T); 342 (G-A)
tRNA-Ser (S2) (tga)	10,148 (−3)	10,213 (−3)	+	66	—	—	—	13	—
NAD1 ^a	11,171 (−3)	10,227 (−3)	−	945	ATA	TAA	314	23	198 (A-G); 231 (G-A)
tRNA-Leu (L1) (tag)	11,261 (−3)	11,195 (−3)	−	67	—	—	—	−26	—
16S rDNA	12,565 (−3)	11,236 (−3)	−	1330	—	—	—	16	—
tRNA-Val (V) (tac)	12,652 (−3)	12,582 (−3)	−	71	—	—	—	−4	—
12S rDNA	13,468 (−3)	12,649 (−3)	−	820	—	—	—	0	—
									Transversions
Control region	13,469 (−3)	14,392 (−12)	+	924 (−9)	—	—	—	0	(two C-T and two T-C)
									Transitions
									(six G-A and one A-G)
tRNA-Ile (I) (gat)	14,393 (−12)	14,459 (−12)	+	67	—	—	—	0	Transversion (T-C)
tRNA-Gln (Q) (ttg)	14,527 (−12)	14,460 (−12)	−	68	—	—	—	1	—
tRNA-Met (M) (cat)	14,529 (−12)	14,597 (−12)	+	69	—	—	—	0	—
NAD2 ^a	14,598 (−12)	15,605 (−12)	+	1008	ATG	TAG	335	−2	26 (T-C)
tRNA-Trp (W) (tca)	15,604 (−12)	15,671 (−12)	+	68	—	—	—	6	—
tRNA-Cys (C) (gca)	15,742 (−12)	15,678 (−12)	−	65	—	—	—	0	—
tRNA-Tyr (Y) (gta)	15,807 (−12)	15,743 (−12)	−	65	—	—	—	0	—

Note: Genes with position (start and stop are highlighted), strand direction [SD] (+: heavy; −: light), length (in bp; deletions/insertions are highlighted in bold), codon (start and stop), length of the amino acid sequence (Laa), number of nucleotides of the intergenic regions (Ign; overlapping regions are highlighted by negative numbers), and difference in the nucleotide sequences (in bold, positions are highlighted in PCGs only and refer to the starting position of the gene). PCGs are reported as standard abbreviations, tRNAs are reported as three-letter and one-letter (as in Figure 2) abbreviations.

^aStart and/or stop positions differ from those predicted by MITOS2.

^bOne different nucleotide (A-G) is present in the intergenic region between NAD4L and tRNA-Thr.

the two species laid in the transition of a single nucleotide. These results and additional features are reported in Table 2. Summarizing, the two mitochondrial genomes showed a similarity value of 99.80% and the differences laid in 20 nucleotides plus the presence of 12 deletions/insertions. The complete mitochondrial genomes were deposited in GenBank

with the accession numbers PV582136 (*C. rissoana*) and PV567534 (*C. granulata*).

3.4. Phylogenetic Analyses. All three nuclear genes were assembled for both *C. rissoana* and *C. granulata*, resulting in sequences of identical lengths (28S: 554 bp; H3: 343 bp;

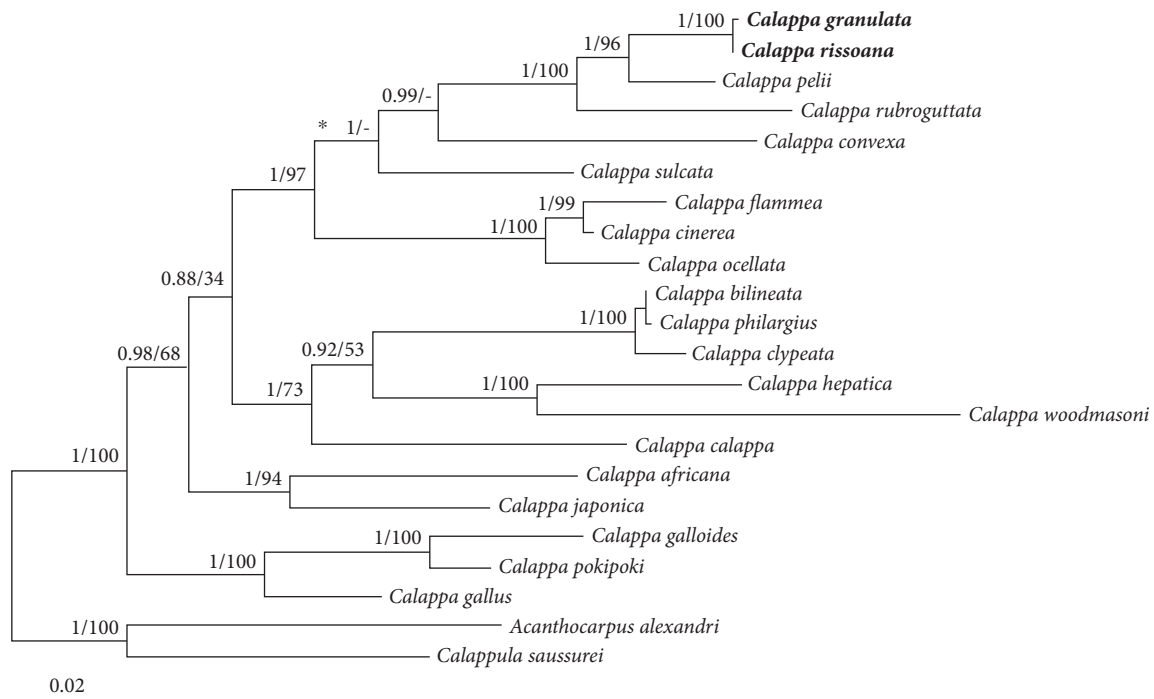


FIGURE 3: Best Bayesian Inference (BI) phylogenetic tree of *Calappa* species based on the five genes (COX1, 16S, 28S, H3, and Enolase) analyzed. Data on the sequences used are reported in the Supporting Information 5 and Supporting Information 6. Material newly sequenced here is reported in bold. Numbers above/below branches represent values of BI posterior probability (pp) and ML bootstrap support (bs). Maximum likelihood bootstrap values are not reported for the clade in which internal relationships among species differed from those recovered with the Bayesian Inference. The scale bar represents nucleotide substitution. The asterisk (*) highlights the clade mentioned in the text which includes *Calappa rissoana* and *Calappa granolata*.

Enolase: 428 bp) and nucleotide sequences between the two species. They were deposited in GenBank with the following accession numbers: PV576371 (*C. rissoana*) and PV576363 (*C. granolata*) for 28S; PV580268 (*C. rissoana*) and PV568316 (*C. granolata*) for H3; PV580267 (*C. rissoana*) and PV568317 (*C. granolata*) for Enolase. A total of 76 sequences, representing 19 taxa (including outgroups), were downloaded from the GenBank database. Additionally, one COX1 sequence (missing in GenBank but corresponding to a taxon already recovered for other genes) was downloaded from BOLD, whereas one RNA-seq dataset (SRR8668051) from the bioproject PRJNA524231 was downloaded from the SRA database, contributing one additional species and raising the number to 20 taxa. The final dataset, thus, included 22 taxa, comprising a total of 92 sequences across five markers (COX1: 21; 16S: 20; 28S: 17; H3: 17; Enolase: 17) with an overall length of 2051 bp (COX1: 656 bp; 16S: 431 bp; 28S: 300 bp; H3: 338 bp; Enolase: 326 bp) (Supporting Information 5 and 6). The best-fit evolutionary models identified were GTR+F+I+G4 for COX1, TPM3u+F+G4 for 16S, K2P+I for 28S, TN+F+I for H3, and TIM2e+I for Enolase. The BI and ML analyses yielded congruent tree topologies, with the exception of a single clade where the internal relationships among species varied, specifically in the placement of *Calappa sulcata* Rathbun, 1898 and *Calappa convexa* de Saussure, 1853 (Supporting Information 5). Hence, the best BI tree with values of posterior probability (pp) and bootstrap support (bs) is shown here (Figure 3). The phylogenetic analyses

identified five major terminal clades, one of which, marked with an asterisk (pp = 1; bs = 40), included both *C. rissoana* and *C. granolata*. These two taxa formed together a monophyletic clade with a robust support (pp = 1; bs = 100) and showed almost null branch lengths.

3.5. Data Mining and Passive Citizen Science Coupled with Statistical Analyses. A total of 337 records were mined from the different sources examined (Supporting Information 7: photos further available on request from the authors). Based on the sizes of the photographed individuals, 37 records were categorized as Class 1 (10.98%), 207 as Class 2 (61.42%), and 93 as Class 3 (27.60%). The highest number of records of atypical individuals was observed in Size Class 1 (26: 70.27% of the class total), followed by Class 2 (33: 15.94% of the class total), while no atypical records were present in Class 3 (Figure 4; Supporting Information 7). Records of typical individuals were distributed across all size classes. Significant differences in the occurrence of atypical characters were also confirmed through PERMANOVA analyses and pairwise tests among all classes (Tables 3 and 4).

Among the records of atypical individuals, the 26 ascribed to Class 1 had the highest percentage of material originating from printed sources (36.54%: 11 records, three of which were also published online). With the exception of one, all these records had been previously published in both papers and books/field guides as *C. tuerkayana* (Supporting Information 7). The majority of the records (27.03%) exhibited a

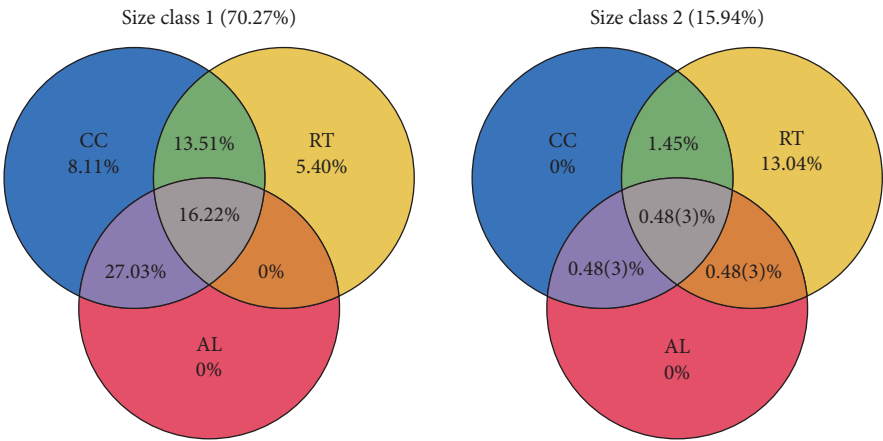


FIGURE 4: Venn diagrams showing the frequency of single and combined atypical characters (CC, RT, and AL) found in individuals of *C. granulata*. Abbreviations: CC, carapace and chelipeds; RT, rostral teeth; AL, ambulatory legs.

TABLE 3: Results of the univariate permutational analyses (PERMANOVA) on the number of atypical characters in each individual of *C. granulata* in the three size classes investigated.

Source	df	MS	Pseudo-F	P(perm)
Cl	2	29.00	106	***
Res	334	0.27	—	—
Tot	336	—	—	—

Note: P(perm), permutational level of probability; Pseudo-F, F critic; Res, residual; Tot, total.
Abbreviations: Cl, size class; df, degrees of freedom; MS, mean squares.
*** $p < 0.001$.

combination of atypical traits in the carapace and legs, followed by individuals showing a combination of all three atypical variables (16.22%). In contrast, records featuring single atypical characters ranked lower (Figure 4). However, a certain degree of atypicality was observed in the investigated variables, with bigger-sized individuals occasionally retaining the atypical bright pinkish-orangish carapace but developing the typical red spots, or displaying atypical pink-orange ambulatory legs, but already turning to the typical yellow-white (e.g., see Figure 5A,B vs Figure 5C). On the contrary, records assigned to Class 2 predominantly originated from web sources, with only five records (12.12%) appearing in printed literature (two of which were also published online). Noteworthy, records from scientific papers consistently identified these specimens as *C. rosea/rissoana*, while those from books/field guides referred to them as *C. granulata* (Supporting Information 7). Also in this class, some individuals showed intermediate color variables between atypical and typical—for example, with atypical bright reddish-orangish carapaces but accompanied by well-developed typical red spots (Figure 5D). However, almost all atypical specimens of Class 2 showed atypical rostral teeth, either alone (13.04%) or in combination with other characters (Figures 4 and 5E). Significant differences in the frequency of atypical characters among the three size classes were also confirmed through the PERMANOVA analyses for all characters, except for the combination rostral teeth + ambulatory legs (Table 5). Pairwise tests further

TABLE 4: Results of the pairwise tests on the number of atypical characters in each of *C. granulata* between the three size classes investigated.

Pairwise comparison	t	P(MC)
1 versus 2	11.30	***
1 versus 3	12.70	***
2 versus 3	3.85	***

Note: P(MC), probability level after Monte Carlo simulations; t, pairwise tests.
*** $p < 0.001$.

revealed a lack of significant differences between Size Classes 2 and 3 across various combinations of characters (Table 6). In addition, results of the Spearman's Rank correlation indicated that there is a significantly large negative relationship between the size class of *Calappa* specimens and the number of atypical traits ($r(335) = -0.51$, $p < 0.001$).

In contrast, 278 (82.49%) records illustrated typical individuals (Figure 5E), the majority of which originated from web sources. Of these, only 29 records (8.99%) were published in scientific papers or book/field guides (eight of which were also published online), all consistently identified as *C. granulata*. Among the records, 11 were ascribed to Size Class 1 (3.96%), 174 to Size Class 2 (62.59%), and 93 to Size Class 3 (33.45%).

4. Discussion

4.1. *Calappa rissoana* Versus *Calappa granulata*: One Single Taxon or Two Related Species?

The recent application of DNA barcoding and, in general, of integrative approaches to the decapod fauna of the north-eastern Atlantic-Mediterranean biogeographic region is finally clarifying the validity and taxonomic relationships between species and within difficult species groups [5, 43]. Building on this, we hereby contribute to the discussion by first addressing the taxonomic status of a box crab species, apparently related to the more common *C. granulata*, which has been considered a Mediterranean endemic species and as such included in international checklists and reference books in the last two decades [7, 35, 45, 46].

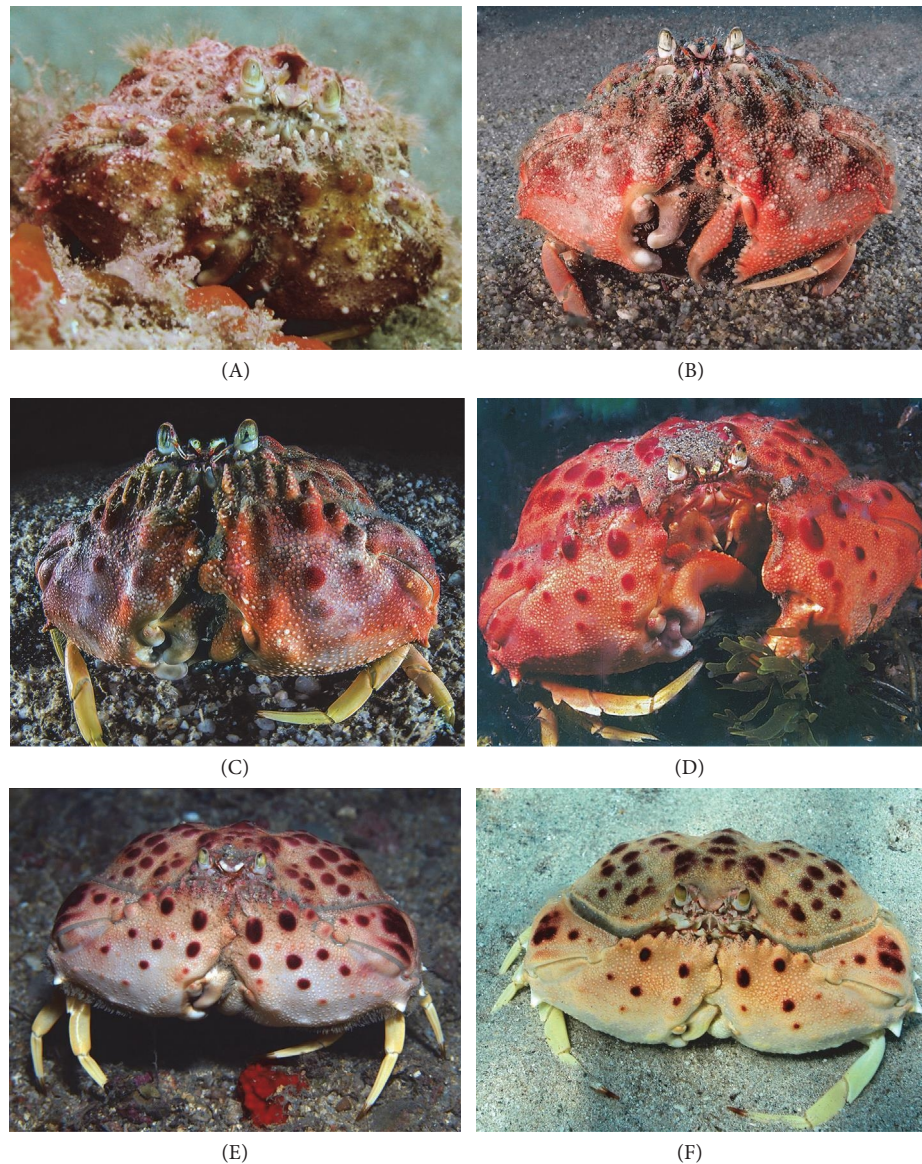


FIGURE 5: Representative color photographs of *C. granolata* mined from printed and web sources and illustrating the different ontogenetic stages that characterize the species. (A–C) *Calappa tuerkayana sensu* Pastore [27]. (A–B) Records 11 and 273 from Capo Noli (Ligurian Sea) (44.19783° N, 8.42708° E). Specimens with three atypical characters. (A) Photo by Ero Tarantino. (B) Photo by Maurizio Pasi. (C) Record 276 from Saline Joniche (Ionian Sea) (37.93803° N, 15.70564° E). Specimens with three atypical characters but already developing the typical red spots on carapace and chelipeds and displaying ambulatory legs turning from the atypical pink-orange to the typical yellow-white. Photo by Mimmo Roscigno. (D–E) *Calappa rissoana sensu* Pastore [27]. (D) Record 301 from Panarea Island (Tyrrhenian Sea) (38.62788° N, 15.07060° E). Specimen with two atypical characters, displaying the atypical reddish carapace and chelipeds with the typical red spots already well-developed. Photo by Francesco Sesso. (E) Record 94 from Cap d'Antibes (western Mediterranean Sea) (43.54186° N, 7.11909° E). Almost a typical specimen, only displaying the atypical white rostral teeth. Photo by Gilles Cavignaux. (F) *Calappa granolata*. Record 68 from La Maddalena Island (Tyrrhenian Sea) (41.20884° N, 9.39320° E). Photo by Antonio Colacino. Specimen displaying all the typical color characters according to the literature.

Notwithstanding its distribution has also been expanded in some studies [47, 48], the general knowledge of the taxon remains scarce so far. This limited information may have stemmed from the ambiguity in Pastore's [15] original description, which contains obscure sentences and measurements, and is also not free from mistakes or inconsistencies, but it is presumably mostly related to the general rarity of

specimens exhibiting the peculiar coloration of *C. rissoana*. Indeed, the species' general reddish carapace background and, most notably, the whitish coloration of the rostral teeth are distinctly visible, even to untrained eyes. Given its distinctive characteristics, if specimens with such features were ever encountered, they would have been likely reported or brought to the attention of marine biologists and decapod specialists

TABLE 5: Results of the univariate permutational analyses (PERMANOVA) on the presence/absence of single and combined atypical characters (CC, RT, and AL) in each individual of *C. granulata* in the three size classes investigated.

Source	df	MS	Pseudo-F	P(perm)
CC				
Cl	2	0.11	13.12	**
Res	334	8.25E-03	—	—
Tot	336	—	—	—
RT				
Cl	2	0.57	7.47	**
Res	334	7.60E-02	—	—
Tot	336	—	—	—
CC + RT				
Cl	2	0.26	12.14	***
Res	334	2.18E-02	—	—
Tot	336	—	—	—
CC + AL				
Cl	2	1.17	47.30	***
Res	334	2.48E-02	—	—
Tot	336	—	—	—
RT + AL				
Cl	2	9.32E-04	0.31	ns
Res	334	2.98E-03	—	—
Tot	336	—	—	—
CC + RT + AL				
Cl	2	0.42	23.08	***
Res	334	1.80E-02	—	—
Tot	336	—	—	—

Note: P(perm), permutational level of probability; Pseudo-F, F critic; Res, residual; Tot, total used.

Abbreviations: AL, ambulatory legs; CC, carapace and chelipeds; Cl, size class; df, degrees of freedom; MS, mean squares; ns, not significant; RT, rostral teeth.

** $p < 0.01$.

*** $p < 0.001$.

over the past decades, even as anomalies or oddities. Consistent with this, the specimen assigned here to *C. rissoana* is the first of this kind that we encountered, despite extensive handling and examining box crabs across various research projects, and such specimens also appear extremely rarely even online, as shown in this study. Aside from these premises, the differences outlined in the literature do not guarantee *per se* the status of a valid species to *C. rissoana*, and the results of this investigation confirm such a suggestion, as the species failed to withstand the integrative taxonomic approach applied here. In fact, except for a few characters, most of the supposed morphological differences were either undetectable or fell within the variability of adult *C. granulata* specimens. However, even those confirmed here, such as the convexity of the carapace, appear to be somewhat labile. Especially in Calappidae, changes in carapace shape may correspond to different survival strategies, with individuals that, upon reaching larger sizes, tend to shift towards muddy and sandy substrates where they bury themselves, with geometric

TABLE 6: Results of the pairwise tests on the presence/absence of single and combined atypical characters (CC, RT, and AL) in each individual of *C. granulata* in the three size classes investigated.

Pairwise comparison	t	P(MC)
CC ^a		
1 versus 2	4.26	***
1 versus 3	2.84	**
RT		
1 versus 2	1.32	ns
1 versus 3	2.29	*
2 versus 3	3.72	***
CC + RT		
1 versus 2	3.90	***
1 versus 3	3.78	***
2 versus 3	1.17	ns
CC + AL		
1 versus 2	8.03	***
1 versus 3	5.82	***
2 versus 3	0.67	ns
CC + RT + AL		
1 versus 2	5.59	***
1 versus 3	4.21	***
2 versus 3	0.67	ns

Note: P(MC), probability level after Monte Carlo simulations; t, pairwise tests.

Abbreviations: AL, ambulatory legs; CC, carapace and chelipeds; ns, not significant; RT, rostral teeth.

^aNo atypical CC alone in Classes 2 and 3.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

adaptations of the carapace that could facilitate this behavior [29, 48]. On the contrary, almost all previously reported color characters for *C. rissoana* were confirmed, with seven of them appearing to be putatively diagnostic. Although color characters are often extremely useful for distinguishing different, but morphologically similar, species [49], these differences may also be due to color morphs, anomalies, intraspecific variations, or even ontogenetic stages. Genetic analyses carried out in this study confirmed the conspecificity between the two species. Sequence length, base comparison, gene content, and mitogenome structure of the two putatively different taxa were consistent with those reported by Lu et al. [21] for *Calappa bilineata* and were nearly identical between them, with dissimilarity values falling well below the barcode gap threshold (about 2%–3%: see Hebert et al. [50]). The absence of genetic differentiation was also observed in nuclear genes, as both the nuclear ribosomal DNA marker and the two nuclear PCGs were identical in the two examined specimens. Furthermore, these similarities were supported both by BI and ML phylogenetic analyses conducted on the concatenated dataset, which consistently merged *C. granulata* and *C. rissoana* into a single taxon. Therefore, this study provides, for the first time, robust evidence demonstrating that the rare endemic species *C. rissoana* (syn. nov.) is another junior synonym of the common and widespread *C. granulata*.

4.2. *What Truly Is “Calappa rissoana”?* Decapods undergo a series of metamorphoses throughout their growth, often involving significant morphological, physiological, and ecological changes [51]. Although the most significant transformations usually occur during the larval phases—a developmental stage extensively studied by researchers worldwide in the recent decades [51, 52]—some decapods continue to experience morphological modifications throughout their lifespan, modifying the shape or ornamentation of specific or entire regions of their body [52]. The transition from the first benthic juvenile stage to adulthood primarily involves structural changes in both internal and external anatomical features [51, 52], but can also include changes in coloration [53]. In this contest, juvenile stages can assume a spectrum of non-adult colors, a phenomenon observed across nearly all major decapod groups—for example, carideans as *Gnathophyllum elegans* (Risso, 1816) [54], anomurans as *Clibanarius virescens* (Krauss, 1843) [55], and brachyurans as *Cancer irroratus* Say, 1817 [56]—constituting a transient polymorphic condition that may be a bet-hedging strategy in a chromatically diverse and unpredictable environment, where visual predators are prevalent [56]. These color changes can easily go unnoticed, as they are often misinterpreted within the broader concepts of intraspecific variability and, more generally, of species-specific polymorphisms [57]. The challenge of maintaining and observing juveniles and subadult individuals in aquariums or confined environments further contributes to this ambiguity, as these modifications typically occur over a longer time-span than larval ones.

The situation regarding *C. granulata* does not differ significantly from that of many other taxa worldwide. The stages from prezoa to the first benthic juvenile have been known for over 30 years [58–60], while the subsequent phase was only recently clarified, with the synonymization of “*C. tuerkayana*” with *C. granulata* [30]. This revision, however, pertained solely to the very early juvenile phases, leaving the morphology and coloration of later stages an open question. That study was also the first to suggest that “*C. rissoana*” might have represented an additional ontogenetic and color stage within the development of *C. granulata*, thus challenging earlier assertions and speculations by other authors. The results obtained here strongly support the former hypothesis, successfully reconstructing all growth phases of *C. granulata* and definitively suggesting that “*C. rissoana*” represents the ontogenetic transition between early juveniles and fully-grown adults. Naturally, as this is a dynamic process, these phases are not always distinctly separated, with typical or atypical traits occasionally appearing at an earlier stage, or even a character manifesting as partially atypical and partially typical, as demonstrated in this study. Notwithstanding this, data obtained through the data mining and passive citizen science approach revealed a progressive shift in coloration occurring in three main phases. The first phase ensures juvenile survival through crypsis, characterized by orangish ambulatory legs and a pinkish–grayish carapace and chelipeds, lacking the typical and well-evident carmine red or dark garnet spots (phase *tuerkayana*). This is soon followed by the second phase, in which the chelipeds and carapace gradually shift in color—

first turning reddish and then orangish—accompanied by the progressive development of white rostral teeth and red spots on both the carapace and chelipeds, while the ambulatory legs remain orangish or transition to yellowish (phase *rissoana*). Finally, the third phase sees the species acquiring the coloration typically recognized and documented for this taxon (phase *granulata*). This conclusion was supported not only by the statistical analyses carried out in this study, which revealed significant and negative correlation in the occurrence of “atypical” color traits at decreasing size classes, but also by a careful re-examination of the color photographs of the material investigated by Innocenti et al. [30]. In particular, larger specimens of “*C. tuerkayana*” were observed developing often imperceptible white rostral teeth and reddish spots on the carapace (Supporting Information 8). Moreover, individual sizes can vary even within the same species due to simple intraspecific variability or environmental factors and food availability, which may have influenced Pastore’s [15] decision to classify these phases as three different species rather than testing the hypothesis that they might instead represent different growth stages of a single species.

The discovery of wide ontogenetic and color changes between juvenile and adult box crabs is relatively new to science and was highlighted only a few years ago by Innocenti et al. [30] in *C. granulata* and by Vossгаetter et al. [61] in *Calappa flammea* (Herbst, 1794). This may explain why early juvenile phases of *C. granulata* were repeatedly described as new varieties or species [38–40], and why the conclusions reached in this study had not been previously hypothesized. Vossгаetter et al. [61] suggested that color plasticity is closely linked to ontogenetic shifts in habitat use, with juveniles inhabiting different environments and adapting their coloration in response to various environmental stressors. Notably, this likely holds true for *C. granulata* as well, as juvenile and sub-adult phases tend to inhabit rocky and coralligenous environments, whereas fully-grown adults predominantly occupy muddy and sandy bottoms. Further research should investigate whether these ontogenetic color changes occur worldwide across box crabs or if this phenomenon is restricted to a few calappid species.

4.3. *Toward the Final Resolution of the Chaotic Mediterranean Box Crab Situation?* Box crabs or shame-faced crabs of the family Calappidae form a diverse group of decapods accounting for more than 120 extant taxa worldwide [35, 62]. They predominantly inhabit tropical and subtropical areas worldwide, with six species also reported from the Mediterranean Sea until a few years ago [7, 15, 29, 30, 63–65]. These were *Cryptosoma cristatum* Brullé, 1837, widespread in the Atlantic Ocean but only known from a single specimen found in the Alboran Sea [63], and five species of the genus *Calappa*, which have been the focus of many faunistic, taxonomic, and interdisciplinary investigations over the centuries, as well as in recent decades (e.g., [15, 30, 42, 44, 65–72]). This number included: (i) *C. granulata*—the type species of the genus *Calappa* by subsequent designation: see Holthuis [16]; (ii) the small *C. tuerkayana* Pastore, 1996, a species described nearly 30 years ago but then rapidly recorded across all the

north-eastern Atlantic-Mediterranean Sea [66–68, 70, 72, 73]; (iii) *C. rosea/rissoana*, the rare endemic taxon investigated here; and (iv–v) two additional species, namely *Calappa hepatica* (Linnaeus, 1758) and *Calappa pelii* Herklots, 1851, that are respectively native to the Indo-West Pacific and the Atlantic Ocean, and have been frequently listed as non-indigenous species in the Mediterranean [64, 65]. Among them, *C. granulata* is the most widespread and one of the most common species in the Mediterranean Sea. This taxon is usually encountered during scuba diving activities on sandy or mixed bottoms, a fact reflected in its frequent documentation through photography and its inclusion in numerous books and field guides illustrating the European and Mediterranean biota (references in the Supporting Information 7). Moreover, *C. granulata* is frequently landed by professional fishermen and used for human consumption both homely and professionally [74, 75]. All this would make it sound like a well-known and generally unmistakable taxon. However, this assumption was already recently mined by Innocenti et al. [30], who addressed a significant gap in knowledge regarding its ontogenetic development and revealed that “*C. tuerkayana*” was actually described based on early juveniles of *C. granulata*. By further synonymizing here also *C. rissoana* with *C. granulata*, we have further reduced the number of native box crabs in the Mediterranean Sea to a single species, thereby challenging even more the previous assumption. By doing so, we have also finally stabilized the nomenclature and taxonomy of *C. rosea* and *C. granulata*, which have remained unsettled for over 200 years. In fact, if *C. granulata* is the only native species inhabiting the Mediterranean Sea, then also *C. rosea* (syn. nov.) can be ultimately regarded as another of its junior synonyms, regardless of the material originally used to describe it and at least until cryptic diversity is eventually found within the older nominal taxon. Furthermore, since the type material of *Cancer granulatus* Linnaeus, 1758 is presumably lost and its original description could theoretically apply to any valid native box crab from the Mediterranean Sea, selecting a neotype to stabilize nomenclature is unnecessary, as all native species previously described from the Mediterranean Sea are now its junior synonyms.

Unfortunately, apart from *C. granulata*, also the three remaining species mentioned above (*Cr. cristatum*, *C. hepatica*, and *C. pelii*) suffer from a high degree of uncertainties, and this may be again partially connected to the present study. *Cryptosoma cristatum* and *C. hepatica* were recorded in single short communications more than two decades ago but have never been found again in the Mediterranean Sea, nor the studies did include photographs or even drawings of the specimens [63, 65]. However, at least the material of the latter species was apparently deposited (without a voucher number) at the Hydrobiological Museum of the Istanbul University (Turkey), potentially allowing for future re-examinations. Given the strong similarities of this species with juveniles of *C. granulata*, a thorough reassessment of this record would be advisable before being accepted in the scientific literature. Similarly, the validity of *C. pelii* has often been debated due to putative similarities with *C. granulata* [76], and this issue has been presumably exacerbated by the paucity of

photographs/drawings available in the literature until very recently. Notwithstanding *C. pelii* is really morphologically and genetically different from *C. granulata* (e.g., see [77]), repeated misidentifications may have occurred also in modern literature, with: (i) the specimen of *C. pelii* figured by De Matos-Pita et al. [78] that closely resembles a small-sized *C. granulata*; (ii) specimens of “*C. pelii*” sequenced by Lobo et al. [79] that showed similarity values of ~99% with *C. granulata*—versus a confirmed difference of more than 5% between the two species: see Ewers-Saucedo et al. [22] and Muñoz et al. [77]; (iii) private sequences in the BOLD System formally ascribed to *C. pelii*, but being different from confirmed ones and again falling within the conspecificity range (99.39%) of *C. granulata*. As for the two species already mentioned earlier in the paragraph, records of *C. pelii* in the Mediterranean Sea are also doubtful, as based on three single and old observations from Italy, Greece, and Spain, none of which was accompanied by photographs, figures, or documented material deposited in any institution [15, 64, 80]. Moreover, at least the material recorded from Italy and Greece is lost (F. Crocetta, unpublished data). Therefore, misidentifications of juveniles or subadults of *C. granulata* may be involved here too.

5. Conclusions

The present study represents the second publication addressing box crab taxonomy in the Mediterranean Sea, confuting the status of an enigmatic and rare endemic species and contributing to a better understanding of the resident decapod biota. Notwithstanding that, further taxonomic and biogeographic studies may still be required to clarify the potential presence of the remaining calappid species in the basin. Given the increasing importance of biodiversity protection and discovery, as well as the need for accurate species assessment, a critical and integrative approach remains essential for scientific progress and must continue to be applied by modern marine biologists, even if studies challenge previous work, statements, and researchers who shaped the historical taxonomy and biogeography of the involved regions.

Data Availability Statement

The sequence data used to support the findings of this study are deposited in GenBank.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This work was supported by the Antitumor Drugs and Vaccines from the Sea (ADVISE), PG/2018/0494374, and National Biodiversity Future Center (NBFC), CN_00000033.

Acknowledgments

Sampling in the Gulf of Naples and literature acquisitions were partially supported by the project Antitumor Drugs and

Vaccines from the Sea—ADViSE (PG/2018/0494374) (FC and VT). The research was supported by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender N. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—Next Generation EU. Project code CN_00000033, Concession-Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP (C63C22000520001) project National Biodiversity Future Center—NBFC (FC). Francesco Mazzella (Ischia Island, Italy) provided the specimen of “*C. rissoana*” investigated here. Domenico Schiano (Ischia Island, Italy) shared connections with several fishermen from Ischia. Ero Tarantino (Italy), Maurizio Pasi (Italy), Mimmo Roscigno (Italy), Francesco Sesso (Italy), Gilles Cavignaux (France), and Antonio Colacino (Italy) provided photographs of the specimens reported in Figure 5. Francesco Tiralongo (Italy) provided photographs of the specimens of “*Calappa tuerkayana*” reported in the Supporting Information 8. Carlo Froggia (Italy) and Sabina Cavicchi (Italy) shed light on the correct publication date of the work of Pastore (1996). Arkadiusz Cegliński, Dominika Mierzwa-Szymkowiak, and Darek Iwan (Museum and Institute of Zoology of Polish Academy of Science - Warsaw, Poland), Laure Corbari and Paula Martin-Lefevre (Muséum National d'Histoire Naturelle - Paris, France), Vladimir Jecmenica (Muséum d'histoire Naturelle de Marseille - Marseilles, France), and Olivier Gerriet (Muséum d'Histoire Naturelle de Nice - Nice, France) provided information on the collections under their care. We are deeply grateful to all of them. This article is part of the Ph.D. project “DAR-ING” (Tackling diversity through DNA barcoding and integrative taxonomy: Decapod Assemblages Revealed IN the Gulf of Naples) of Valentina Tanduo.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information 1. Summary of the principal works addressing the studied calappid species, with a focus on the taxonomic features and nomenclatural notes concerning the investigated taxon.

Supporting Information 2. Diagnostic characters observed in the box crab specimens examined here and final morphological identifications.

Supporting Information 3. Color and morphological features of *Calappa rissoana* and *Calappa granulata*.

Supporting Information 4. Multi-dimensional scaling (MDS) plots of the morphological traits investigated here.

Supporting Information 5. GenBank and BOLD sequence data for *Calappa* species included in the phylogenetic analyses, with the resulting Bayesian Inference and maximum likelihood phylogenetic trees.

Supporting Information 6. Alignment of concatenated gene sequences used in phylogenetic analyses.

Supporting Information 7. List of photographic records of *Calappa granulata* mined from printed and web sources.

Supporting Information 8. Color photographs of selected material of “*Calappa tuerkayana*”.

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