



Evaluating the taxonomic status of the large sized *Tricolia* Risso, 1826 in the Northeast Atlantic and Mediterranean Sea

Lara Baptista^{a,b,c,d,e,*}, Giulia Fassio^f, Serge Gofas^{g,h}, Marco Oliverio^f, Sérgio P. Ávila^{a,b,c,d,e,i}, António M. Santos^{c,e,j}

^a CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, 9501-801 Ponta Delgada, Açores, Portugal

^b MPB-Marine Palaeontology and Biogeography Lab, University of the Azores, Rua da Mãe de Deus, 9501-801 Ponta Delgada, Açores, Portugal

^c BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

^d UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, R. Mãe de Deus 13A, 9500-321 Ponta Delgada, Portugal

^e Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 1021/1055, 4169-007 Porto, Portugal

^f Dipartimento di Biologia e Biotecnologie ‘Charles Darwin’, Sapienza Università di Roma, Viale dell’Università 32, I-00185, Rome, Italy and NBFC, National Biodiversity Future Center, Palermo 90133, Italy

^g Departamento de Biología Animal, Facultad de Ciencias, Universidad de MálagaE-29071 Málaga, Spain

^h Muséum National d’Histoire Naturelle, Paris, France

ⁱ Departamento de Biologia, Faculdade de Ciências e Tecnologia, Universidade dos Açores, 9501-801 Ponta Delgada, Açores, Portugal

^j CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, no. 7, 4485-661 Vairão, Portugal

ARTICLE INFO

Keywords:

Phylogeny
Sympatric occurrence
New species
Atlantic archipelagos
Oceanographic barriers
Taxonomic revision

ABSTRACT

Despite a long history of taxonomic studies on the genus *Tricolia* Risso, 1826, there is a shortfall on thorough systematic molecular reviews of the taxon from the NE Atlantic and Mediterranean coasts. Aiming to assess the genetic distinctness among morphospecies and the taxonomic status of currently accepted large sized species in these areas, we conducted a molecular phylogenetic analysis of the genus based on one mitochondrial (*cox1*) and two nuclear (*28S* and *ITS2*) markers. Seven *Tricolia* species were consistently retrieved in the analyses, including a new genetic lineage in the NE Atlantic designated as *Tricolia* sp. 1. Molecular analyses revealed that only one species, *T. azorica*, occurs in the NE Atlantic archipelagos. The sister taxa *T. pullus* (Mediterranean) and *T. picta* (NE Atlantic) should be classified as distinct species, instead of subspecies of the *T. pullus* group (sensu Gofas 1982). *Tricolia miniata* is also a complex of species in the Mediterranean and future studies across the distribution range are necessary to clarify its status.

1. Introduction

The genus *Tricolia* Risso, 1826 is the representative of the subfamily Tricoliinae (pheasant shells, Phasianellidae family) in the Old World and Australasia. Although a revision of South African *Tricolia* species has been recently produced (Nangammbi et al., 2016), most of the taxonomic studies on the genus focus on taxa inhabiting the eastern Atlantic and Mediterranean coasts, where highly variable and closely interrelated forms occur (Gofas, 1982, 1986, 1993). Such reviews were mainly based on shell polychromatism, radular features, and sympatric occurrence of discrete morphologies (Gofas, 1982, 1986), which were used to create a dichotomous key for the classification of *Tricolia* species, later

updated with notes on external soft parts (Gofas, 1993). Despite extreme variation in shell coloration and patterns, the importance of polychromatism in the characterization of taxa when correlated with other characters was pointed out by Gofas (1982, 1986). Still, it should be used carefully to distinguish species, as globose shells with a white to creamy background color and darker red stripes and/or dots seem to be shared by all *Tricolia* species (Gofas, 1982). *Tricolia* is distinguishable from other Trochoidea by its white calcareous operculum (Gofas, 1982). *Tricolia pullus* (referred to as *T. pullus sensu lato*) is the type species of the genus, represented by the currently recognized nominal subspecies *T. pullus pullus* (Linnaeus, 1758) at its type locality, the “Mediterranean Sea”.

* Corresponding author at: CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, 9501-801 Ponta Delgada, Açores, Portugal.

E-mail addresses: larabaptista@hotmail.com, lbaptista@cibio.up.pt (L. Baptista).

<https://doi.org/10.1016/j.ympev.2023.107857>

Received 16 August 2022; Received in revised form 6 June 2023; Accepted 8 June 2023

Available online 13 June 2023

1055-7903/© 2023 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

As in all other members of the superfamily, sexes are separate and produces non-feeding planktonic larvae. For instance, *T. pullus* (Linnaeus, 1758) larvae survive in the water column for a maximum of 68 h after fertilization and become benthic thereafter (Manly, 1976). Assuming that all *Tricolia* species have a short larval stage, their ability for dispersal is expected to be reduced, a feature which may constrain their geographical distribution range, and deeply impact connectivity among populations (Modica et al., 2017; Scheltema, 1995, 1986). Dispersal of non-planktotrophic marine larvae and juveniles is still possible by several mechanisms (for a review see Winston, 2012), of which rafting in floating substrata is the most significant to overcome oceanic barriers in temperate waters (Ávila, 2013; Ávila et al., 2019; Winston, 2012). *Tricolia* species possess characteristics that increase their chances to disperse through rafting, namely their small size and their habitat usually among marine vegetation (Thiel and Gutow, 2005a, 2005b; Thiel and Hays, 2006). The biogeographical processes operating in the NE Atlantic and Mediterranean are complex, frequently promoting differentiation of species with short-lived larvae at different spatial and temporal scales (Pascual et al., 2017). Of particular interest is the genetic diversity of *Tricolia* across the NE Atlantic archipelagos, as a recent review of the biogeographical affinities by Freitas et al. (2019) distinguished the Azores and Webbsnesia ecoregions. A geographic structure, with intraspecific variation between Atlantic and Mediterranean populations is expected, as well as an influence of habitat continuity, oceanographic fronts, and sea-surface temperatures in the Mediterranean in shaping the biogeographical of *Tricolia* species (Marzouk et al., 2017; Pascual et al., 2017).

Gofas (1982, 1993) thoroughly evaluated the *Tricolia* species occurring in the Eastern Atlantic and Mediterranean shores (see Table 1 for details), typically inhabiting shallow-water rocky infralittoral areas, either associated with seaweeds or with seagrasses such as *Posidonia*, *Cymodocea*, and *Zostera*. Four morphs/subspecies of *T. pullus sensu lato*, all with an average size of 5–10 mm, are recognized across the whole geographic range (sensu Gofas, 1982): 1) *T. pullus pullus* (Linnaeus, 1758); 2) *T. pullus picta* (da Costa, 1778); 3) *T. pullus azorica* (Dautzenberg, 1889); 4) *T. pullus canarica* (Nordsieck, 1973). Other species are recorded in the Eastern Atlantic Ocean: *T. algoidea* (Pallary, 1920), *T. miniata* (Monterossato, 1884), and *T. petiti* (Craven, 1882). In the Mediterranean Sea, the number of recognised *Tricolia* species is higher (Gofas, 1982, 1993): *T. tenuis* (Michaud, 1829), *T. speciosa* (Muhlfeldt, 1824), *T. tingitana* Gofas, 1982, *T. deschampsii* Gofas, 1993,

Table 1

Distribution range of *Tricolia* species occurring in the Eastern Atlantic Ocean and Mediterranean Sea, following Gofas (1982, 1993).

	Species	Distribution range
Large sized	<i>T. pullus pullus</i> (Linnaeus, 1758)	Mediterranean basin
	<i>T. pullus picta</i> (da Costa, 1778)	British Isles to Morocco
	<i>T. pullus azorica</i> (Dautzenberg, 1889)	Azores
	<i>T. pullus canarica</i> (Nordsieck, 1973)	Madeira, Canaries
	<i>T. miniata</i> (Monterossato, 1884)	Morocco, Algeria, Mediterranean Southern Spain
	<i>T. petiti</i> (Craven, 1882)	Gulf of Guinea
	<i>T. tenuis</i> (Michaud, 1829)	Mediterranean Sea
	<i>T. speciosa</i> (Muhlfeldt, 1824)	Mediterranean Sea, Black Sea
	<i>T. algoidea</i> (Pallary, 1920)	Atlantic Moroccan coast
	<i>T. tingitana</i> Gofas, 1982	Strait of Gibraltar
Small sized	<i>T. deschampsii</i> Gofas, 1993	Strait of Gibraltar and Alboran platform
	<i>T. entomocheila</i> Gofas, 1993	Strait of Gibraltar and Canary Islands
	<i>T. nordsiecki</i> (Talavera, 1978)	Strait of Gibraltar and Selvagens Islands
	<i>T. punctura</i> Gofas, 1993	Corsica, Mediterranean coast of France
	<i>T. landinii</i> Bogi and Campani, 2007	Sicily and SE Spain

T. entomocheila Gofas, 1993, *T. nordsiecki* (Talavera, 1978), and *T. punctura* Gofas, 1993. Bogi and Campani (2007) described yet another Mediterranean species from the shallow rocky shores of Sicily – *T. landinii* – later re-described by Scuderi and Reitano (2012). The larger sized species – *T. pullus sensu lato*, *T. miniata*, *T. petiti*, *T. speciosa*, and *T. tenuis* – constitute a stable taxonomic group of well-known species easily recognized. Yet, the taxonomic status of some subspecies and morphs (e.g. *T. pullus azorica* and *T. pullus canarica*) is still regarded as dubious (Scuderi and Reitano, 2012).

Recently, *T. pullus* was included in large-scale molecular phylogenies of Vetigastropoda to ascertain the systematic position of the family Phasianellidae (Williams and Ozawa, 2006; Williams et al., 2008). Nangambi et al. (2016) conducted a phylogenetic analysis of South African taxa, producing sequence data for several *Tricolia* species occurring in that region. Nonetheless, despite the early interest for *Tricolia*, a thorough systematic review of the genus on the NE Atlantic and Mediterranean coasts based on molecular markers is still lacking. Therefore, we hereby propose a molecular phylogenetic analysis of the large sized species of *Tricolia* in the NE Atlantic and Mediterranean waters, aiming to characterize the molecular diversity among morpho-species, assess the taxonomic status of currently accepted species and subspecies, and to check the informativeness of morphological characters as diagnostic features in the genus.

2. Material and methods

2.1. Sample collection

A total of 135 specimens of the genus *Tricolia* were included in this study, assigned to a nominal morphospecies based on the most recent taxonomy of the group (Gofas, 1982, 1986, 1993), relying on characters of the teleoconch, as follows:

- Twenty-four specimens from six islands of the Azores, identified as *T. pullus azorica* (Dautzenberg, 1889);
- Five specimens (two from Madeira, two from Selvagens, one from the Canaries), identified as *T. pullus canarica* (Nordsieck, 1973);
- Seventeen specimens (eleven from Northern Portugal and six from Brittany, France), identified as *T. pullus picta* (da Costa, 1778);
- Nine specimens (two from the Mediterranean coasts of Spain, two from Tunisia, five from Corsica) identified as *T. pullus pullus* (Linnaeus, 1758);
- Seven specimens (one from the Mediterranean coasts of Spain, six from Corsica) identified as *T. miniata* (Monterossato, 1884);
- Twenty-two specimens (two from the Mediterranean coasts of Spain, 20 from Corsica) identified as *T. tenuis* (Michaud, 1829);
- Twenty-seven specimens (16 from Tunisia, two from Italy, nine from Corsica) identified as *T. speciosa* (Muhlfeldt, 1824).

Then, 34 additional specimens (from Brittany, Portugal, Mediterranean Spain, Corsica, Italy, Tunisia, Greece, Cyprus) were not identified with certainty to the species level either because they were juveniles, due to their equivocal morphology or because the very small voucher shell was broken during tissue extraction (see Table S1 for details).

Fresh samples of *Tricolia pullus azorica* were obtained by integral algal scraping on subtidal habitats (15–25 m) by scuba-diving in the Azores in July of 2018 and 2019: specimens were preserved in 96% ethanol and deposited in the Marine Molluscs Collection of the Department of Biology of the University of the Azores (DBUA). Samples of *T. pullus azorica* from additional Azorean locations and of *Tricolia* spp. from the Iberian Peninsula and Western Mediterranean Sea were obtained after a thorough search in DBUA and CIBIO-InBIO mollusc collections. *Tricolia pullus pullus*, *T. miniata*, and *T. tenuis* individuals were retrieved from the collection constituted by Serge Gofas at the University of Malaga. *Tricolia* specimens from the North Atlantic and Central Mediterranean Sea were made available by the malacological collection

of the Department of Biology and Biotechnologies “Charles Darwin”, Sapienza University of Rome (BAU), as well as by the Molluscs Collection of the Muséum National d’Histoire Naturelle, Paris (MNHN-IM). Details regarding the samples used in this study are provided in Fig. 1 and Table S1.

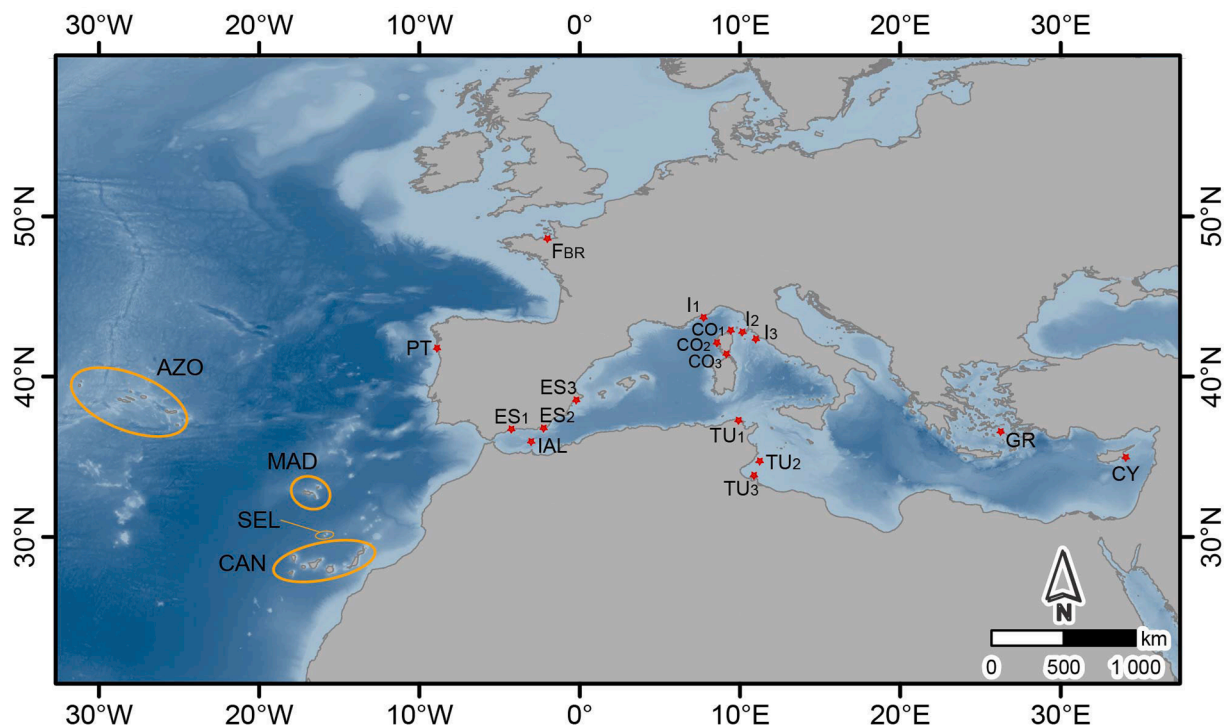
2.2. Laboratory procedures

DNA extraction was performed with tissue retrieved from the foot or entire animal with the commercial EasySpin® Genomic DNA Tissue Kit (Citomed, Lisbon, Portugal), following the manufacturer’s protocol or following a ‘salting-out’ protocol (Aljanabi and Martinez, 1997). PCR reactions were performed in 25 µl volumes, entailing 3 µl of DNA, – 10 × buffer MgCl₂ free, 2.5 mM MgCl₂, 0.2 mM dNTP, 10 µM of each primer, 0.1 µg µl⁻¹ bovine serum albumin (BSA, Promega) and 0.3 U Platinum Taq DNA polymerase or of BIOTAQ DNA polymerase. PCR amplifications were conducted using primers sets and cycling conditions presented in Table 2. Successful PCR products were purified and bidirectionally sequenced at GENEWIZ’s Sanger Sequencing Service, Azenta Life Sciences company (Leipzig, Germany). A total of 56 *cox1* sequences were produced at the Service de Systématique Moléculaire (UMS 2700 2AD, MNHN, CNRS).

2.3. Analyses of molecular datasets

A manual check of misreads in chromatograms was performed with BioEdit v.7.0.5.3 (Hall, 1999). Sequences of the mitochondrial coding *cox1* gene were translated into amino acids with ExPASy Translate Tool to detect stop codons and pseudogenes. GenBank accession numbers for newly generated sequences are listed in Table S1, as well as those for publicly available *cox1* and *28S* sequences of *T. pullus* and *T. pullus azorica* included in the datasets. Representatives of the family Phasiacellidae were chosen as outgroups for the phylogenetic reconstructions: *Hiloe variabilis* (Pease, 1861) (AB365219, AM048723) for the analysis with *cox1*, *28S*, and concatenated dataset; *Agathistoma viridulum* (Gmelin, 1791) (AY68209) for the *ITS2* phylogeny.

The *cox1* dataset was aligned with Clustal Omega (Sievers et al., 2011), whereas the *28S* and *ITS2* datasets were aligned with MAFFT v7 online server (Kuraku et al., 2013; Katoh et al., 2019). Default settings were used to align the *28S* dataset, whilst the Q-INS-I iterative method was set for *ITS2* to account for secondary structures. Datasets were reduced to haplotypes using the web-based software ALTER (Glez-Peña et al., 2010). GBLOCKS Server v0.91b (Castresana, 2000) was used to eliminate poorly aligned positions and divergent regions of the *28S* and *ITS2*, as these often hinder the phylogenetic signal (Talavera and Castresana, 2007). Estimates of the raw (*p*) distances (i.e., proportion of



Caption:

Localities - NE Atlantic

AZO - Azores
CAN - Canaries
MAD - Madeira
SEL - Selvagens
PT - North of Portugal
FBR - Brittany, France

Localities - Mediterranean Sea

CO1 - N Corsica, France
CO2 - S Corsica, France
CO3 - W Corsica, France
CY - Cyprus
ES1 - Malaga, Spain
ES2 - Almería, Spain
ES3 - Benidorm, Spain
GR - Astypalea, Greece
IAL - Alborán Island, Spain
I1 - Liguria, Italy
I2 - Elba Island, Italy
I3 - Tuscan Archipelago, Italy
TU1 - Menzel Abderrahmen, Tunisia
TU2 - Kerkennah Island, Tunisia
TU3 - Djerba Island, Tunisia

Bathymetry (m)

0
< -4500

Fig. 1. Study area in the Northeast Atlantic and Mediterranean Sea. Sampled archipelagos are delimited by orange circles, whereas sampled coastal sites are indicated by red stars. Coastline delimitation according to available data from the Portuguese Hydrographic Institute and bathymetry derived from GEBCO 2020. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

Marker, primer name and direction, sequence (5'-3'), reference and cycling conditions used (temperature, time, and the number of cycles).

Marker/ Primer	Sequence (5'-3')	Reference	Cycling conditions
cox1			
LCO1490 (F)	GGTCAACAAATCATAAAGATATTGG	(Folmer et al., 1994)	94 °C 4'; [x35] 94 °C 40';
COR722b (R)	TAAACTTCAGGGTGACCAAAAAATYA	(Wilke and Davis, 2000)	55 °C 1'; 72 °C 1'; 72 °C 4'
lgLCO1490 (F)	TITCIACIAAYCAYAARGAYATTGG	(Geller et al., 2013)	
lgHCO2198 (R)	TAIACYTCIGGRTGICCRARAAYCA		
28S			
C1' (F)	ACCCGCTGAATTAAGCAT	(Hassouna et al., 1984)	94 °C 4'; [x35]
D2 (R)	TCCGTGTTTCAAGACGG		94 °C 30'; 58–60 °C 30'; 72 °C 50'; 72 °C 4'
ITS2			
ITS-3d (F)	GCATCGATGAAGAACGCAG	(Oliverio and Mariottini, 2001)	94 °C 4'; [x35] 94 °C 30'; 60 °C 40'; 72 °C 45'; 72 °C 4'
ITS-4r (R)	AGTTTCTTTTCTCCGCTTA		

fixed differences between two sequences) among *Tricolia* species were performed in MEGA11 (Tamura et al., 2021), with one representative of each species per locality sampled. The analysis was performed considering all codon positions after exclusion of positions containing gaps and/or missing data for each sequence pair analyzed. Parsimony informative sites in the nuclear datasets were checked with MEGA11.

2.4. Species delimitation and phylogenetic analyses

An iterative approach to species delimitation was used (Yeates et al. 2011), where species are considered as hypotheses to be subsequently tested by independent evidences (Puillandre et al. 2009, 2012a,b). Morphological identification of the specimens represented the starting point to identify putative morpho(sub)species. These morphology-based Preliminary Species Hypotheses (PSH) were contrasted with the results of a series of analyses of the molecular datasets.

Haplotype subnetworks at the 95% connection limit often coincide with Linnaean species-level names, constituting a useful tool to delineate putative species-boundaries using nucleotide datasets in poorly known groups (Hart and Sunday, 2007; Chen et al., 2010). Therefore, statistical parsimony haplotype networks based on individual *cox1* sequences were generated with the software TCS v1.21 (Clement et al., 2000), regardless of species category, to enlighten the relationships among samples. The program tcsBU (Santos et al., 2016) was used to annotate the resulting haplotype networks, facilitating the visualization of intricate relationships. One sample arbitrarily chosen from each haplotype subnetwork was used in a search for matching sequences in the Barcode of Life Data System v3 database (BOLD; Ratnasingham and Hebert, 2007), in February 2023.

Then, PSH were tested against a molecular approach, with two methods based on pairwise genetic distance on the *cox1* dataset: Automatic Barcode Gap Discovery (ABGD; Puillandre et al., 2012a) and Assemble Species by Automatic Partitioning (ASAP; Puillandre et al., 2021). The ABGD approach sorts sequences into hypothetical species based on the barcode gap, which is modelled based on the pairwise genetic distance of the dataset in test and priors of intraspecific divergence (Puillandre et al., 2012a); the latter was adjusted to 0.2 in this

analysis. The ASAP method follows a hierarchical clustering algorithm based solely on pairwise genetic distances and species hypothesis are proposed without biological prior information of intraspecific diversity, thus providing unbiased estimates (Puillandre et al., 2021).

Two tree-based approaches to species delimitation were also employed: the Bayesian implementation of the Poisson Tree Process model (bPTP; Zhang et al., 2013) and the multi-rate PTP (mPTP; Kapli et al., 2017). In PTP approaches, speciation events are inferred based on the number of substitutions (Zhang et al., 2013). As a recent improvement, the mPTP process incorporates intraspecific divergence caused by different evolutionary histories or uneven sampling of the species (Kapli et al., 2017). The bPTP analysis was conducted for 10^5 MCMC generations, a thinning of 100, and 25% burn-in. Default settings were applied to the mPTP approach.

Finally, species hypotheses retained after the ABGD analysis were tested for their reciprocal monophyly by performing phylogenetic analyses on single-gene alignments (*cox1*, *ITS2*, and *28S*) and on a concatenated dataset (*cox1* + *ITS2* + *28S*). The determination of the best-fit partitioning schemes and models of molecular evolution, using Akaike's Information Criterion (Akaike, 1973), was conducted with PartitionFinder v1.1.1 (Lanfear et al., 2017). To minimize the saturation effect and allow for heterogeneous rates of evolution in the *cox1* marker (Pond et al., 2009; Salemi, 2009), data partitioning by codon was defined as follows: GTR-G (1st partition), GTR + I + G (2nd partition), and GTR + I (3rd partition) in the dataset including *H. variabilis*; GTR + G (1st and 2nd partitions), GTR + I (3rd partitions) for the analyses without the outgroup. For the *28S* dataset, the GTR model was set with the outgroup *H. variabilis*. The model HKY + I was set for the *ITS2* dataset with *Agathistoma viridulum* as the outgroup, and GTR + I for *ITS2* partition in the concatenated dataset. Bayesian Inference (BI) and Maximum Likelihood (ML) methodologies were used to reconstruct the phylogenies, using MrBayes v3.2.7 (Ronquist et al., 2012) and W-IQ-Tree web server (Trifinopoulos et al., 2016), respectively. For the BI, two independent runs with four chains each for 2×10^7 generations, sampling of trees and parameters every 1000 generations, and heating parameter of 0.25 were set. When the average standard deviation of split frequencies was lower than 0.01, stationarity was considered to have been reached. Both runs were used to estimate majority-rule consensus trees, with a 25% burn-in. ML analyses were run for 1000 ultrafast bootstraps (UFBoot; Hoang et al., 2018), SH-aLRT branch tests (Guindon et al., 2010) for 1000 replicates, and partitioned models (Chernomor et al., 2016) for the *cox1*. The final trees were visualized with FigTree v1.4.3, setting the outgroup or rooting according to the ancestral split of lineages.

3. Results

The alignment of the *cox1* dataset comprised 137 sequences with a total length of 658 bp. The *28S* dataset comprised 39 sequences with a maximum of 809 bp, after quality control and trimming to match in size, whereas the *ITS2* dataset included 24 sequences with lengths ranging from 407 to 426 bp.

3.1. Species delimitation and phylogenetic analyses

The TCS network of the *cox1* dataset (Fig. 2) distinguished a total of 11 haplotype networks at 95% confidence level. Samples morphologically assigned to *T. miniata*, *T. tenuis*, and *T. speciosa*, formed individual clusters as expected for each nominal species. *Tricolia miniata* was, however, separated in four subclusters, according to the geographic origin of the samples (Corsica, Malaga, Benidorm, and Alboran Island). Individuals from the Atlantic archipelagos, morphologically assigned to the nominal groups *T. pullus azorica* (Azores) and *T. pullus canarica* (Madeira, Selvagens, and Canaries), could not be distinguished, with most of the Azorean haplotypes displayed in a star-like shape. Samples from the Northern Portugal were assigned to two genetic lineages:

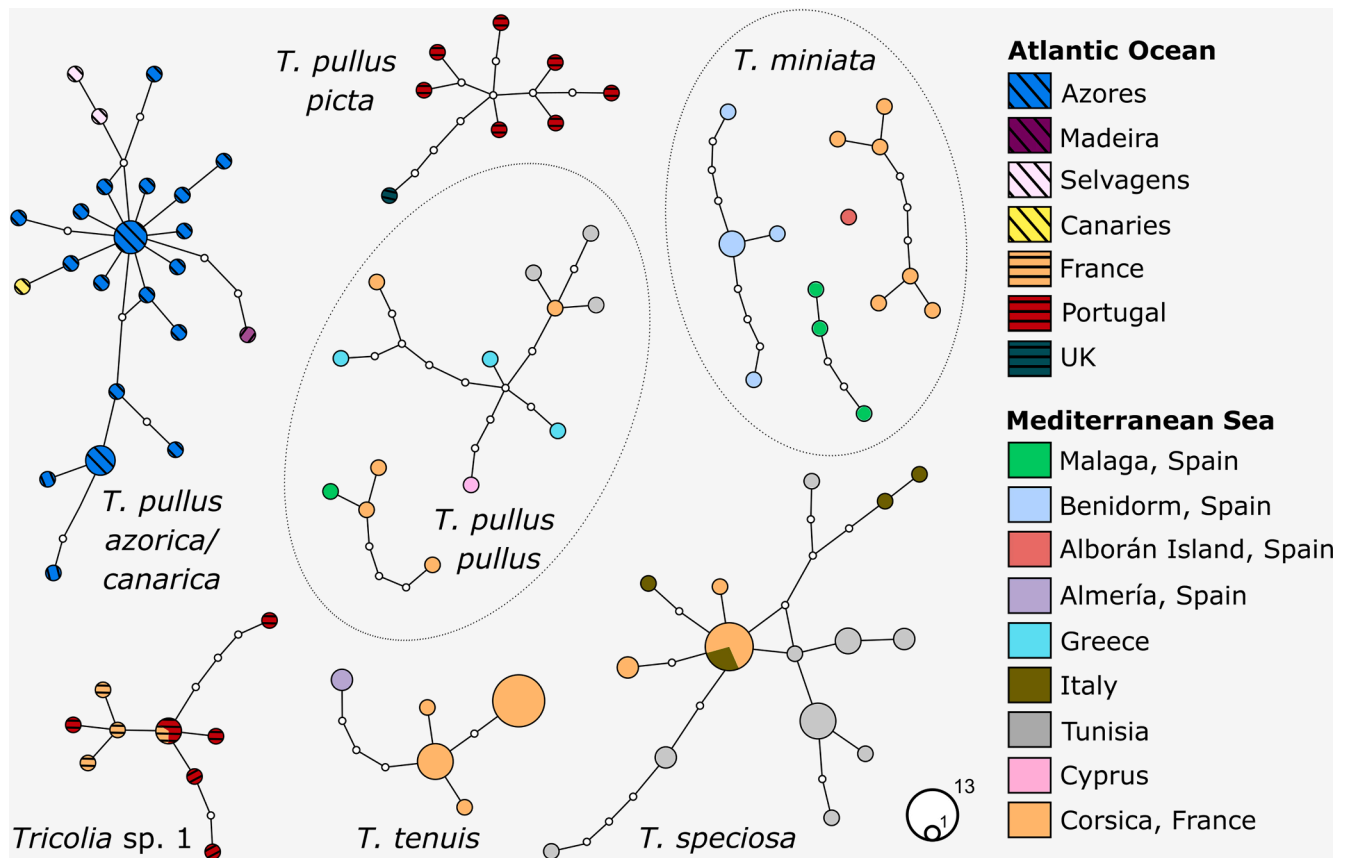


Fig. 2. Eleven haplotype networks of *cox1*, at 95% parsimony connection limit, for 137 sequences/86 haplotypes of the *Tricolia* dataset. If the samples were identified to the species level, the cluster is labelled accordingly. Color code according to the geographical origin of the samples. The size of the circles is proportional to the frequency of each haplotype; small uncolored circles represent non-observed haplotypes; each line connecting haplotypes represents a single mutational change. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Tricolia sp. 1 together with samples from Northern France, and *T. pullus picta* with samples from the UK. *Tricolia pullus pullus* was divided into two distinct haplotype networks.

In a BOLD search, as of February 2023, all queries were assigned to *Tricolia* BINs. Our search revealed the existence of three distinct BINs of *Tricolia* taxa: 1) *T. pullus azorica*, from the Azores (BOLD: AAY7593); 2) *T. pullus sensu lato* from UK (BOLD: AAW8158) and private sequences identified as '*T. pullus canarica*' from Galicia, Spain; 3) private sequences identified as '*T. pullus azorica*' from Galicia, Spain. *Tricolia* sequences from the Azores and Northern Portugal (SMA3582, MIN4645, and MIN4651) were assigned to different BINs with similarity levels > 98%. The results of these searches in relation to the three recognized BINs were summarized in Table 3.

In the species delimitation analyses (Figs. 3-4), ABGD and ASAP estimated ten species hypotheses, as also did bPTP, whilst mPTP estimated nine. For the distance-based approaches, the number of subsets identified prior to the recursive analyses was six in the ABGD and nine in the ASAP. The species hypothesis were concordant in the four approaches, with the following exceptions: bPTP and mPTP failed to distinguish *Tricolia* sp. 1 as a putative species but it was consistently retrieved with ABGD and ASAP; bPTP and mPTP separated *T. pullus pullus* from the Western (Tunisia, Greece, Cyprus) and Eastern Mediterranean (Malaga, Corsica); mPTP clustered *T. miniata* from Malaga and Benidorm as the same taxon whereas the other three methodologies distinguished each of the four Spanish localities as unique taxa.

We obtained phylogenetic reconstructions based on individual markers (Fig. 3, Fig. S1-3) and on the concatenated datasets (Fig. 4). For each individual marker, BI and ML inferences produced comparable topologies for all the major splits. Ultrafast bootstrap values are more

Table 3

BOLD matches of *Tricolia azorica* (SMA3582, Azores), *T. picta* (MIN4645, Portugal) and *Tricolia* sp. 1 (MIN4651, Portugal) to three *Tricolia* BINs in the database, as of February 2023. The highest similarity match for each query is highlighted in bold.

Query	Taxon match	BOLD BIN	% similarity
<i>Tricolia azorica</i> (SMA352)	<i>Tricolia pullus azorica</i>	BOLD: AAY7593	98.46–98.77
	' <i>Tricolia pullus azorica</i> ' (private)	–	92.78–93.08
	<i>Tricolia pullus</i> + <i>'Tricolia pullus canarica'</i> (private)	BOLD: AAW8158-	83.7783.46–83.77
<i>Tricolia picta</i> (MIN4645)	<i>Tricolia pullus azorica</i>	BOLD: AAY7593	84–85.5
	' <i>Tricolia pullus azorica</i> ' (private)	–	84–85.5
	<i>Tricolia pullus</i> + <i>'Tricolia pullus canarica'</i> (private)	BOLD: AAW8158-	99.0899.24–99.39
<i>Tricolia</i> sp. 1 (MIN4651)	<i>Tricolia pullus azorica</i>	BOLD: AAY7593	94.73–95.24
	' <i>Tricolia pullus azorica</i> ' (private)	–	99.2–99.39
	<i>Tricolia pullus</i> + <i>'Tricolia pullus canarica'</i> (private)	BOLD: AAW8158-	85.47–85.78

unbiased than the traditional bootstraps, and thus we have considered a clade to likely be real when SH-aLRT \geq 80% and Ufboot \geq 95%, as suggested by IQ-TREE authors (Minh et al., 2013).

The relationships inferred with the *cox1* phylogeny (see Fig. 3, Fig. S1) confirmed as reciprocally monophyletic almost all species hypotheses emerged from the distance-based and tree-based delimitation

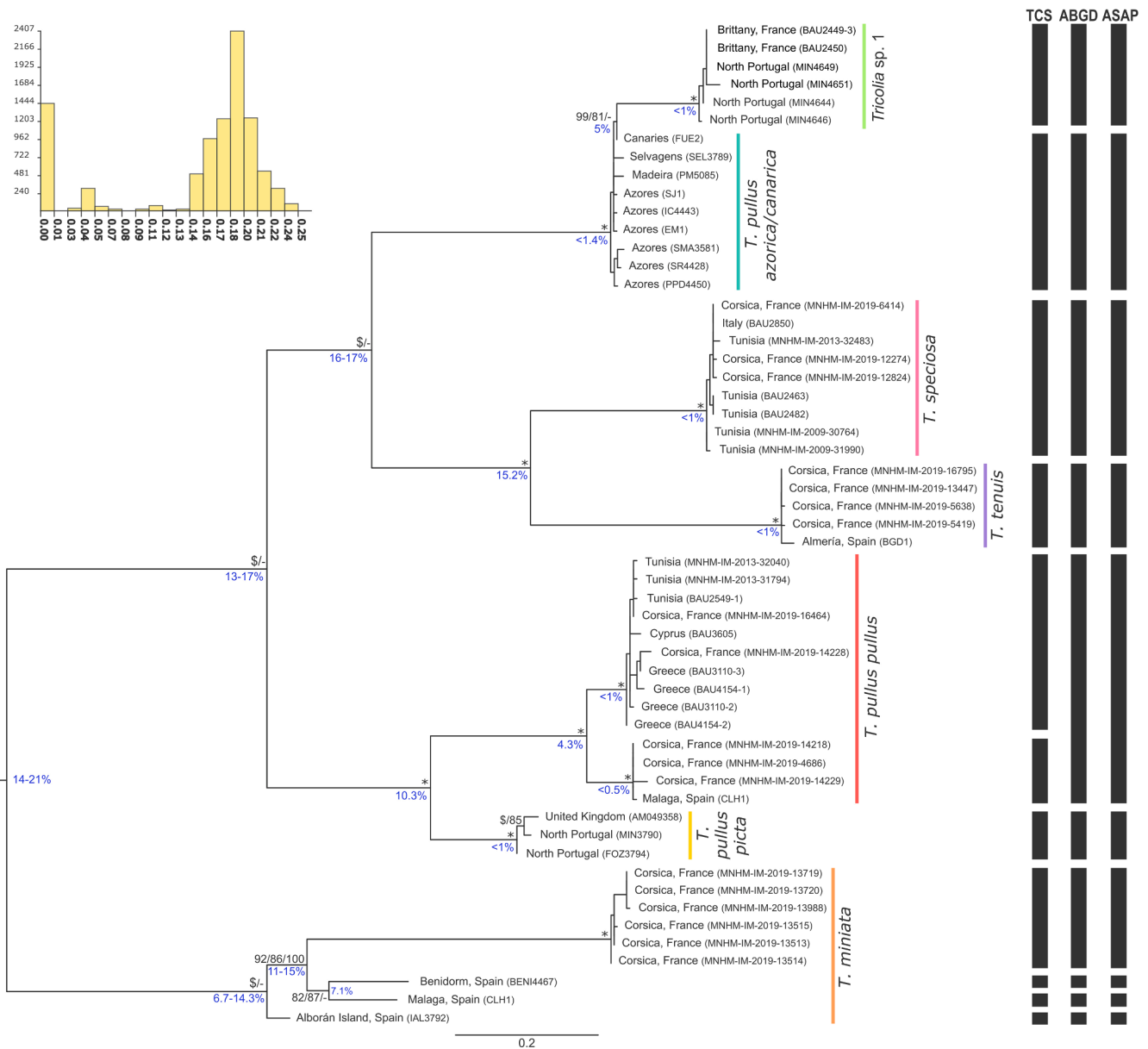


Fig. 3. Maximum Likelihood tree and species delimitation approaches based on the mitochondrial *cox1* gene. Values at the nodes correspond to branch tests (SH-aLTR), ultrafast bootstrap support (Ufboot), and posterior probability (PP). Hyphen (-) indicates nodes absent in one of the phylogenetic reconstructions; asterisk (*) indicates nodes supported by both ML and BI analyses (SH-aLTR \geq 80%, Ufboot \geq 95%, PP \geq 95%); dollar (\$) indicates nodes supported only in the ML reconstruction (SH-aLTR \geq 80%, Ufboot \geq 95%). The histograms graph portrays the distribution of the pairwise genetic divergence among the assayed specimens. Mean divergence levels among *Tricolia* terminals are depicted at the nodes in blue. *Tricolia* species are indicated with different colored shading. Geographical origins and identification of the specimen representing each *Tricolia* haplotype are given as terminal labels. Scale bar represents substitutions per site. Haplotype network and distance-based species delimitation results are depicted by black rectangles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

methods. The only exception was represented by the insular Atlantic specimens that did not show as a monophyletic *T. pullus azorica/canarica* clade (sequences were paraphyletic in a major clade along with a sub-clade *T. sp. 1*).

With the nuclear markers (Fig. S2-3) support values at relevant nodes were often lower and polytomies arose, shadowing detailed intraspecific relationships. In particular, insular *Tricolia* and *Tricolia sp. 1* were not distinguished as reciprocally monophyletic. Moreover, *T. pullus pullus* and *T. pullus picta* were positioned as sister taxa in the 28S reconstruction, but undistinguishable with the *ITS2* marker. Nevertheless, there was concordance between the major genetic groups inferred with mitochondrial (Fig. 3, Fig. S1) and nuclear (Fig. S2-3) markers.

Overall, relationships in the concatenated phylogeny (Fig. 4) were

well-resolved and strongly supported by both ML and BI methodologies. *Tricolia* specimens from the Azores, Madeira, Selvagens, and Canaries were pooled as a single taxon, with moderate phylogenetic support. Insular *Tricolia* and continental Atlantic *Tricolia sp. 1* from the Portuguese and French Atlantic coasts form a clade as sister-taxa, well supported by ML but not in the BI phylogeny. The second major clade showed intricate relationships among the Mediterranean and Atlantic *T. pullus (sensu lato)*, together with the Mediterranean *T. miniata*. Within *T. pullus*, two clades (Western Mediterranean and Central-East Mediterranean) were distinguished. *Tricolia pullus picta* was represented by individuals from Portugal and the UK. *Tricolia miniata* included three lineages spread across the sampled Spanish localities. The resulting concatenated tree demonstrated that NE Atlantic taxa as currently

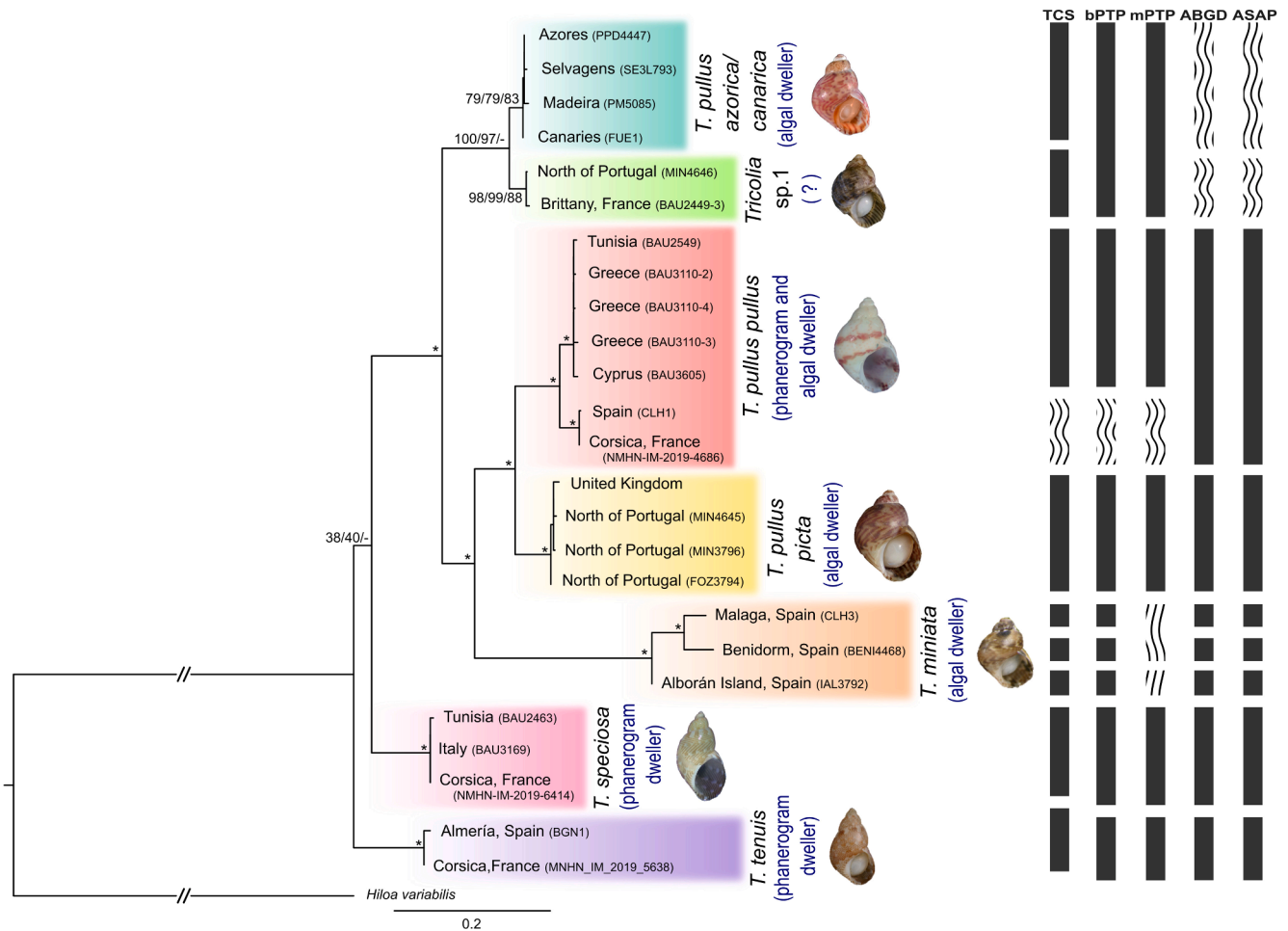


Fig. 4. Maximum Likelihood tree of the concatenated dataset (*cox1* + *28S* + *ITS2*). Values at the nodes correspond to branch tests (SH-aLTR) values, ultrafast bootstrap support (Ufboot), and posterior probability (PP). Hyphen (-) indicates nodes absent in one of the phylogenetic reconstructions; asterisk (*) indicates nodes supported by both ML and BI analyses (SH-aLTR \geq 80%, Ufboot \geq 95%, PP \geq 95%). *Tricolia* species are indicated with different colored shading; preferred substrates of each species is indicated in blue between brackets. Shell images are merely illustrative, not to scale. Geographical origins and identification of the specimen representing each *Tricolia* haplotype are given as terminal labels. Scale bar represents substitutions per site. Results of species delimitation approaches are depicted by black rectangles; wavy-lines indicate discordance of the species hypothesis proposed by different approaches. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

conceived are not monophyletic, with the occurrence of two genetic lineages on the northern shores of Portugal. Finally, the Mediterranean *T. speciosa* and *T. tenuis* branch earlier, although their position was poorly supported.

3.2. Sequence data

Raw (p) distances between 22 *cox1* haplotypes, selected to represent every locality sampled by species (Table S2a), revealed a mean divergence level of 15.5% (9.4 – 18.8%) among *Tricolia* species from the Atlantic and the Mediterranean Sea. On average, *Tricolia* specimens from the Azores, Selvagens and the Canaries diverged by less than 1.5%. Two genetic lineages diverging 14.3% nucleotide differences were detected in the North of Portugal: i) one closely related to *T. pullus sensu lato* from the United Kingdom, and referred to as *T. pullus picta*; ii) the other one, more closely related to samples from France, designated as *Tricolia* sp. 1. Two *T. miniata* lineages from the Western Mediterranean Sea and Alborán Island are distinguished with differentiation levels up to 7.3%. Two genetic groups – Western Mediterranean and Central-East Mediterranean – were present within *T. pullus pullus* with distances ranging from 0.2% to 4.6%. Finally, *T. tenuis* and *T. speciosa* diverge by 15.1%, each forming a consistent group with low intra-specific divergence levels.

The *28S* dataset, reduced to 15 haplotypes (Table S2b), distinguished six genetic groups as follows: 1) *Tricolia* from the Atlantic archipelagos and *Tricolia* sp. 1; 2) *T. pullus picta* from Northern Portugal and the UK; 3) *T. pullus pullus* from the Mediterranean; 4) *T. miniata*; 5) *T. tenuis*, and 6) *T. speciosa*. Raw (p) distances ranged from intraspecific levels of 0% to interspecific divergence of 1.5% between *T. speciosa* and *T. miniata*. For the *ITS2* marker, nucleotide differences between 14 haplotypes ranged from 0 to 6.4% (Table S2c). Insular *Tricolia* were undistinguishable and diverged from *Tricolia* sp. 1 by less than 0.5%. *Tricolia pullus picta* and *T. pullus pullus* are only differentiated by 0.5–0.8%. *Tricolia tenuis* holds the most different *ITS2* sequence, diverging 5.2–6.4% from the other congeners. Despite the low power of the nuclear markers to distinguish *Tricolia* taxa, nine parsimony informative sites were detected along the 809 bp *28S* alignment and 32 were present in the 428 bp *ITS2* dataset.

4. Discussion

4.1. Species assignment and marker performance

The specimens assayed genetically were initially assigned to five morphospecies, of which one not corresponding to a known nominal taxon (*Tricolia* sp. 1), and one split into four traditional subspecies (*Tricolia pullus pullus*, *T. p. azorica*, *T. p. canarica*, and *T. p. picta*). The

species delimitation process adopted, while broadly confirmed the groups suggested by the morphological approach, led to significant redefinition of the status and relationships among them.

The analysis of the *cox1* dataset with the TCS program revealed 11 networks (Fig. 2) across the Mediterranean and NE Atlantic samples, at the 95% connection limit, which may represent a starting point in the identification of putative species (Hart and Sunday, 2007; Chen et al., 2010). Specimens not identified to the species level ended up in those haplotype networks, and their subsequent inclusion in phylogenetic reconstructions allowed confirmation of their identification. TCS networks broadly corresponded to morpho(sub)species, with a few exceptions: *Tricolia* samples from the Atlantic Archipelagos could not be distinguished as either *T. pullus azorica* or *T. pullus canarica*; conversely,

two subnetworks of *T. pullus pullus*, and four of *T. miniata* were detected.

Species delimitations with different methodologies (distance-based and tree-based) were overall concordant. Discordance in a few groups (e.g. distinction of *Tricolia* sp. 1 from insular *Tricolia*) can be related to intrinsic limitations of the methods: PTP methods are sensitive to uneven sampling of the species and non-strictly monophyletic groups, and do not account for divergent intraspecific variation across groups, whilst distance-based methods do not account for evolutionary relationships among the samples (Kapli et al., 2017). Whenever a species hypothesis is suggested by haplotype network analysis or species delimitation approaches, further testing and validation with independent geographical, ecological, and phylogenetic data are required (Chen et al., 2010; Puillandre et al., 2021, 2012b). An integrative interpretation of these

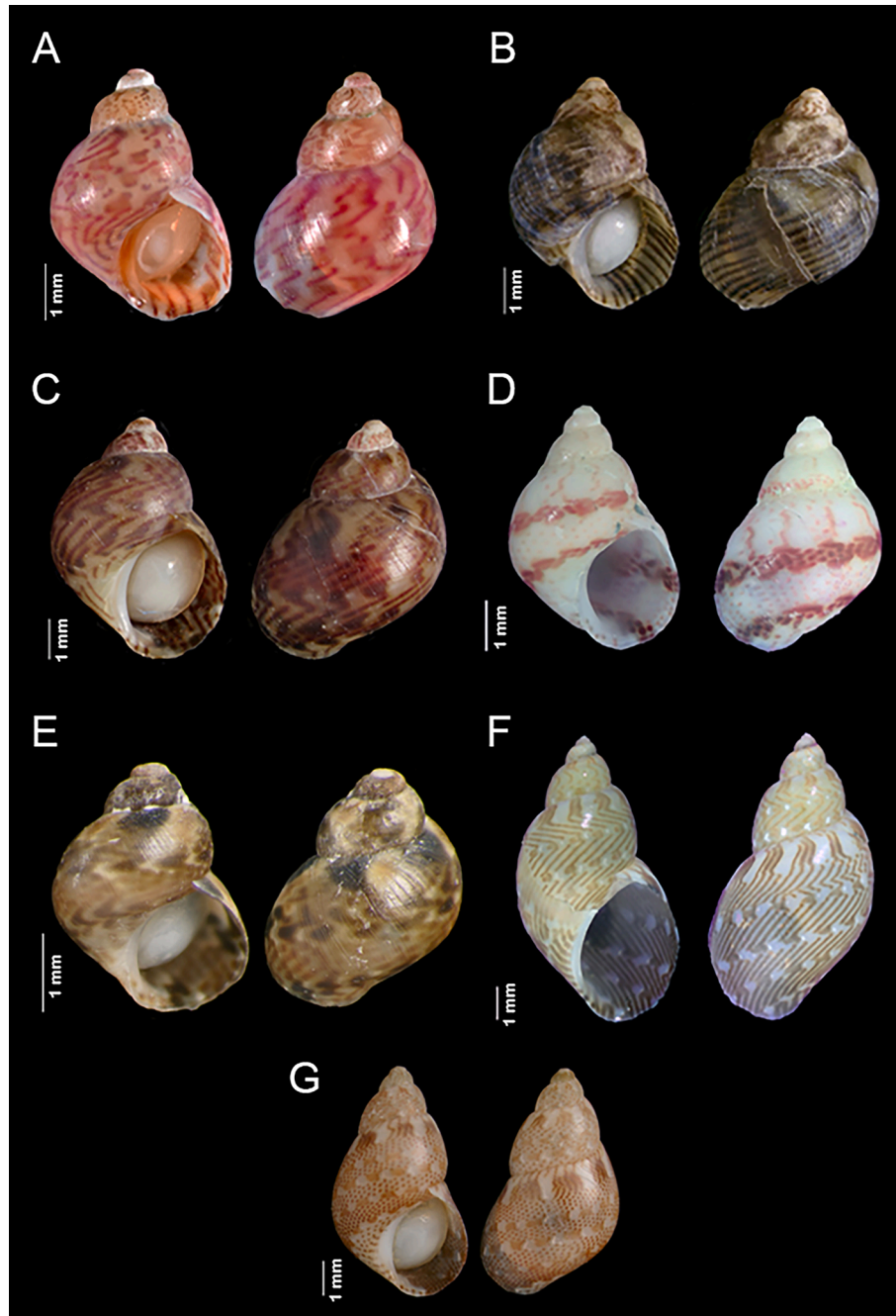


Fig. 5. *Tricolia* species from the NE Atlantic (A-C) and Mediterranean (D-G). A) *T. azorica* from Sabrina Seamount (Azores, Portugal); B) *Tricolia* sp. 1 from Northern Portugal; C) *T. picta* from Northern Portugal; D) *T. pullus* from Astypalea Island (Greece) (BAU-3110-2); E) *T. miniata* from Calahonda (province of Málaga, Spain); F) *T. speciosa* from Gallinara Island (Italy) (BAU-3582); G) *T. tenuis* from Bahía de los Genoveses (province of Almería, Spain).

results was conducted for each *Tricolia* species in the following sections of this study, particularly to evaluate the status of the various lineages, including *Tricolia* sp. 1.

For most clades, similar clustering patterns were obtained in the *cox1*, *28S*, and *ITS2* phylogenies, suggesting the absence of processes potentially affecting the mitochondrial phylogeny, namely introgression and incomplete lineage sorting (Ballard and Whitlock, 2004; Quinteiro et al., 2011). Despite the low levels of nuclear divergence, recent demographic expansion seems to have contributed to a higher mitochondrial differentiation (Després, 2019), as suggested by the star-like subnetwork inferred for the insular *T. azorica*. The nuclear *28S* sequences appear to be slow markers, without insertions/deletions or structural differences, as expected for nuclear markers (Borges et al., 2012; Fassio et al., 2019; Vieira et al., 2019). However, distinction was possible among the major genetic groups and species, including between *T. pullus pullus* and *T. pullus picta* to some extent. The nuclear *ITS2*, despite being a faster-evolving marker than *28S* and considered a valuable tool for mollusk phylogenetics and taxonomy (Oliverio et al., 2002; Puillandre et al., 2011; Fassio et al., 2019, 2021), had no significant resolution to differentiate *T. pullus pullus* and *T. pullus picta*, with overall poorly supported relationships, but revealing the expected insertion/deletions among different species.

The analyses of species delimitation, haplotype network and phylogenetic reconstructions, generally agreed with the species assignments previously described. In summary, a total of seven large sized taxa were eventually recognized in this study: *T. azorica*, *Tricolia* sp. 1, and *T. pullus picta* in the NE Atlantic (Fig. 5 A-C, respectively); *T. pullus pullus*, *T. speciosa*, *T. tenuis*, and *T. miniata* in the Mediterranean Sea (Fig. 5 D-G, respectively). Following this study, we update Gofas (1982, 1993) regarding the distribution ranges and main morphological characteristics of large sized *Tricolia* (except *T. petiti*, not included in this study), which are summarized in Table 4.

4.2. The status of *Tricolia* species in the NE Atlantic

In recent works dealing with NE Atlantic marine mollusks, two *Tricolia* subspecies have been recognized in the archipelagos: *T. pullus azorica* endemic to the Azorean Islands, and *T. pullus canarica* occurring further south in Madeira, Selvagens, and Canaries (Gofas, 1982, 1986; Segers et al., 2009; Cordeiro et al., 2015; Freitas et al., 2019). Our results revealed a high genetic similarity between these two forms of *Tricolia*, suggesting that only one genetic lineage occurs in the NE Atlantic

Table 4
Distribution and habitat usage of large sized *Tricolia* species, updated following the results of this study. Dubious reports are indicated with a question mark (?).

Species	Distribution range	Habitat usage
<i>T. pullus pullus</i> (Linnaeus, 1758)	Western and Eastern Mediterranean basins	Phanerogram and algal dweller; infralittoral.
<i>T. pullus picta</i> (da Costa, 1778)	British Isles to Morocco	Algal dweller; infralittoral in moderately sheltered to exposed localities.
<i>T. azorica</i> (Dautzenberg, 1889)	Azores, Madeira, Canaries,	Algal dweller; infralittoral in moderately surfed localities.
<i>T. miniata</i> (Monterosato, 1884) species complex	Atlantic Moroccan coasts, Western Mediterranean basin, Alboran Sea, Corsica, Malta (?)	Algal dweller; intertidal spring tides in the Atlantic; shallow infralittoral in the Mediterranean.
<i>T. tenuis</i> (Michaud, 1829)	Mediterranean, Cabo Verde (?)	Phanerogram dweller; infralittoral in sheltered localities.
<i>T. speciosa</i> (Muhlfeldt, 1824)	Mediterranean (except Alboran Sea), Black Sea	Phanerogram dweller; lower infralittoral.
<i>Tricolia</i> sp. 1	North Portugal, Brittany (France)	Preferred habitat unknown; so far found in the shallow infralittoral associated to seaweeds.

archipelagos, inhabiting seaweeds. This finding matches the biogeographical expectations of species distributions in the recently defined Webnesia ecoregion (Freitas et al., 2019). Inter-archipelagic connectivity can potentially be maintained under the current system established in the NE Atlantic as reported for other marine invertebrates (Sá-Pinto et al., 2008; Baptista et al., 2021a; Quinteiro et al., 2020; Vieira et al., 2022). The Azores and Canary Currents facilitate rafting episodes and thus dispersal of non-planktonic organisms among the Atlantic archipelagos, as this constitutes the primary mechanism of long-distance transport across deep waters in the open ocean (Ávila, 2006; Thiel and Haye, 2006; Ávila et al., 2019). Given the morphological and genetic evidence together, we demonstrate that all *Tricolia* from the Atlantic archipelagos (Fig. 5A) are not a subspecies of *T. pullus* and, instead, the nominal species *Tricolia azorica* (Dautzenberg, 1889) should be used for them.

Our data revealed the existence of two distinct lineages of *Tricolia* in the European Atlantic coasts with a considerable degree of genetic differentiation among them [14% (*cox1*), 0.2% (*28S*), 1.4% (*ITS2*)]. The potential occurrence of two morphological variants in the NE Atlantic coasts had already been suggested by Monterosato, 1889, who distinguished *Eudora dubia* Monterosato, 1889 and *Eudora picta* (da Costa, 1778) on the coasts of Morocco. One of the two genetic lineages observed corresponds to the group identified as *T. pullus picta*. It includes a specimen sampled from southern UK (Wembury, Plymouth) used as outgroup by Williams and Ozawa (2006) in the phylogenetic study of the family Turbinidae. This particular specimen can be regarded as the representative of NE Atlantic *Tricolia* which was formally described as *Turbo pictus* by da Costa, 1778 from Cornwall and Exmouth (Devonshire). According to recent reference works (Hayward and Ryland, 2017; Wigham and Graham, 2017) no other *Tricolia* species are known for the UK. Like for the insular species *T. azorica*, results from species delimitation analyses (bPTP, mPTP), *cox1* haplotype networks, and genetic distances estimates with the Mediterranean *T. pullus* [10% (*cox1*), 0.1% (*28S*)] suggest that the lineages called *T. pullus pullus* and *T. pullus picta* should rather be regarded as distinct species, and should be named *T. pullus* (Linnaeus, 1758) and *T. picta* (da Costa, 1778), respectively. Morphologically, *T. picta* has whorls more flattened in their upper part and reddish flames under the suture with wavy oblique patterns on a translucent whitish to brownish background (Fig. 5C). So far, there is no evidence that this species enters the Mediterranean. Although the work of Monterosato, 1889 registered the occurrence of two putative *Tricolia* species (listed under the genus *Eudora*) in Morocco, one cannot assign one of them to *T. picta* without additional information because there is at least another *Tricolia* lineage in the Atlantic coast of continental Europe.

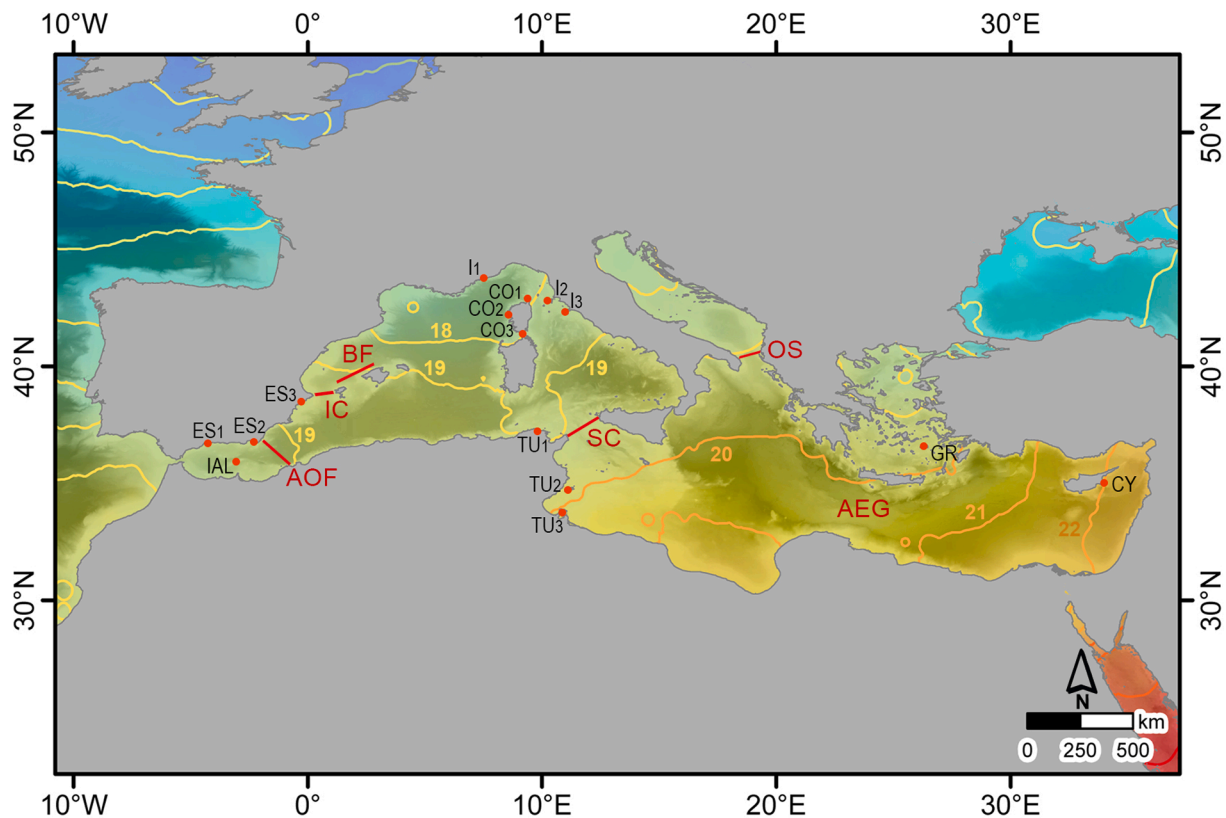
The new lineage, designated as *Tricolia* sp. 1 (Fig. 5B), shows more genetic affinity with the insular *T. azorica* than with the sympatric species *T. picta*. It was found to occur from Northern Portugal to Brittany (France) in seaweeds. Additional sampling is necessary to determine whether the species also occurs with phanerograms or if it is an obligate algal dweller as the sister species *T. azorica*. Divergence with insular forms reaches 5% for *cox1* and less than 1% for the nuclear markers. While tree-based species delimitation analyses could not distinguish *Tricolia* sp. 1 from the group of *T. azorica*, *cox1* haplotype networks and distance-based species delimitation analyses suggested it is a distinct species. Considering the morphological distinction and the allopatric occurrence in distinct biogeographical units (sensu Freitas et al., 2019) we hereby support the distinctiveness of *Tricolia* sp. 1 as an independent genetic lineage. A formal recognition as a species requires further characterization of its ecology and distribution. Interestingly, evidence for this “new” *Tricolia* genetic lineage had already been described but was probably overlooked. Borges et al. (2016) found that their *Tricolia* specimens from Azores matched with two distinct BINs in BOLD Systems v3 database. One of the BINs corresponded to the sequence AM049358 from Wembury, UK (84.6% similarity), which is a representative of *T. picta* as discussed before. The other BIN contained private sequences from Galicia (Spain) which differed only 5% from the Azorean

specimens, a differentiation level which is compatible with the divergence found between *T. azorica* and *Tricolia* sp. 1 in the present work.

As for other species with non-planktotrophic larvae, rafting should be the most important mechanism for long-distance dispersal in *Tricolia* (Manly, 1976; Scheltema, 1995, 1989, 1986; Winston, 2012). A possible scenario for the differentiation of *Tricolia* sp. 1 would involve an exceptional dispersal event of *T. azorica* back to Europe and subsequent differentiation. The complex dynamics of sea-surface circulation of the North Atlantic subtropical gyre (see Baptista et al., 2021a for details), and separation by deep waters reduce the connectivity between the archipelagos and continental coasts, explaining the ongoing differentiation of mainland and insular populations. Whatever the true status of *Tricolia* sp. 1 may be, moderate levels of genetic differentiation between the Atlantic archipelagos and the mainland have been observed for many marine species with or without planktonic larvae (e.g. Sá-Pinto et al., 2008; Desiderato et al., 2019; Freitas et al., 2019; Riesgo et al., 2019; Vieira et al., 2019; Quinteiro et al., 2020; Baptista et al., 2021a,b).

4.3. The status of *Tricolia* species in the Mediterranean

Four Mediterranean species of *Tricolia* were included in this study – *T. pullus*, *T. miniata*, *T. speciosa*, and *T. tenuis* (Fig. 5D-G) – and all seem to co-occur around Corsica and in SE Spain. The mitochondrial differentiation levels probably reflect recent processes of divergence related to oceanographic discontinuities or sea-surface temperatures (SST) in the Mediterranean (Fig. 6) (Sá-Pinto et al., 2012; Marzouk et al., 2017; Pascual et al., 2017). These can trigger some genetic differentiation but are less relevant for gene flow in species with short-lived larvae and reduced adult mobility such as *Tricolia* (Manly, 1976; Pascual et al., 2017). Continuous spatial distribution of suitable infralittoral habitat facilitates gene flow, as seems to occur in *T. pullus* and *T. tenuis* from the Southern Spanish coasts and Corsica (Ligurian Sea). These two species are associated with the phanerogams *Posidonia oceanica* (L.) Delile, 1813 and *Cymodocea nodosa* (Ucria) Ascherson, 1870, respectively, which, despite the current declining trend, are still estimated to cover



Caption:

Oceanographical fronts

AEG - Aegean Front
AOF - Almería-Orán Front
BF - Balearic Front
IC - Ibiza Channel
OS - Otranto Strait
SC - Sicily Channel

Localities

CO1 - N Corsica, France
CO2 - S Corsica, France
CO3 - W Corsica, France
CY - Cyprus
ES1 - Malaga, Spain
ES2 - Almería, Spain
ES3 - Benirdorm, Spain
GR - Greece

Localities

IAL - Alborán Island, Spain
I1 - Liguria, Italy
I2 - Elba Island, Italy
I3 - Tuscan Archipelago, Italy
TU1 - Menzel Abderrahen, Tunisia
TU2 - Kerkennah Island, Tunisia
TU3 - Djerba Island, Tunisia

Fig. 6. Major oceanographical fronts and mean Sea Surface Temperatures (SST) in the Mediterranean Sea. Isotherms in Celsius degrees (°C) in the Mediterranean Sea are labelled at a 1 °C scale; isotherms outside of the study area are not considered. Acronyms in red correspond to oceanographical fronts, whereas black to localities sampled; details available in the caption. Coastline delimitation according to available data from the Portuguese Hydrographic Institute, background digital elevation model generated from GEBCO 2020, and mean SSTs from COBE-SST2 data provided by the NOAA/OAR/ESRL PSL (Hirahara et al., 2014). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1.2 million ha of the Mediterranean basin (Gubbay et al., 2016; Telesca et al., 2015). The influence of the oceanographic fronts of Almeria-Oran (AOF) and Ibiza Channel (IC) (Fig. 6) in the connectivity between the Alboran and Ligurian Seas seems to be negligible in these species. Taxonomic remarks and biogeographical considerations for each species are provided over the next paragraphs.

Tricolia pullus is commonly found in Mediterranean infralittoral *Posidonia* meadows down to 40 m depths. With considerable levels of genetic differentiation from *T. picta* [10% (*cox1*), 0.1% (28S)] and *T. azorica* [14% (*cox1*), 0.5% (28S), 0.3% (*ITS2*)], plus distinct morphological features (see Gofas, 1982), we believe these three taxa to represent good species and should be distinguished as such, instead of subspecies of the *T. pullus* group (Gofas, 1982). Thus, the nominal *T. pullus* (Linnaeus, 1758) should be recovered. The differentiation between the Atlantic and Mediterranean forms is not uncommon in marine invertebrates, associated to oceanographical breaks and/or historical processes (see Patarnello et al., 2007; Marzouk et al., 2017; Pascual et al., 2017; Riesgo et al., 2019 and references therein). The phylogeographic break between the Western and Eastern Mediterranean basins (Fig. 3) is associated with the oceanographical phenomena located at the Sicily Channel (SC, Fig. 6) (Marzouk et al., 2017; Sá-Pinto et al., 2012, 2010 and references therein), which hamper interpretations of the biogeographical affinities of populations in the area. In our dataset, Eastern Tunisian populations of *T. pullus* are closely related to Eastern Mediterranean localities (Greece and Cyprus), likely associated to local sea-surface circulation patterns and the potential role of the Aegean Front (AG; Fig. 6) (Sá-Pinto et al., 2012; Marzouk et al., 2017; Pascual et al., 2017).

In the case of *T. miniata* (Fig. 5E), only samples from the Western Mediterranean area were analyzed (Fig. 6). Yet, our results suggested that this lineage comprises a species complex, as mitochondrial divergence between specimens ranged from 7 to 15% but differences were negligible at the nuclear level. Species complexes are common among gastropods and have lately been the focus of integrative taxonomic approaches, as is the case of *Ocenebrina aciculata* (Lamarck, 1822), *Talisman scrobilator* (Linnaeus, 1758), *Stramonita haemastoma* (Linnaeus, 1767), *Dendropoma petraeum* (Monterosato, 1884), to name a few (Calvo et al., 2009, 2015; Claremont et al., 2011; Barco et al., 2018; Smriglio et al., 2019; Crocetta et al., 2020). The high levels of divergence in the *cox1* among localities within the Alboran Sea (Malaga, Benidorm, and Alboran Island) were striking. The formation of the AOF and two gyres contribute to a complex surface circulation in the basin (see Muñoz et al., 2015; Pascual et al., 2017 for details). Whereas the first is likely to explain the genetic break between Malaga and Benidorm (Patarnello et al., 2007; Pascual et al., 2017), the geographical location of Alboran Island between the West and East Alboran Gyres and deep waters separating it from mainland enhance the geographical and temporal isolation of insular populations (Urra et al., 2013). As conchological material from Alboran Island and Benidorm was destroyed during DNA extraction, we only tentatively identify these specimens as *T. miniata*. The high differentiation between Corsican and Spanish specimens matches the expectations for different Mediterranean ecoregions, as defined by Spalding et al. (2007), and the possibility that the Corsican lineage represents an ongoing process of cryptic speciation cannot be fully discarded (Calvo et al., 2009; Sá-Pinto et al., 2012; Vieira et al., 2019).

Tricolia miniata is reported from the Atlantic Moroccan coast and Strait of Gibraltar, as well as from the Mediterranean southern Spain, coast of Algiers, Corsica (France), Palermo (Italy), and Malta (Gofas, 1982). With sea-surface temperatures increased by 3–4 °C (Fig. 6), Malta's habitats are predicted to be unsuitable for *Halopteris scoparia* Sauvageau, 1904 (Gamliel et al., 2020), the preferential habitat of *T. miniata* (Gofas, 1982). In fact, the only report of *T. miniata* in Maltese waters is from Monterosato (1884) and it is unlikely to occur there nowadays. Unfortunately, we did not have access to Atlantic *T. miniata* specimens, although their inclusion in future works will be of utmost

importance to confirm whether they should be assigned to different nominal groups.

Tricolia speciosa inhabits infralittoral *Posidonia* seagrasses in the Mediterranean (Fig. 5F). In our dataset, it was represented by closely related specimens from Corsica, Italy, and Tunisia. The low levels of differentiation among localities matched the genetic similarity of *Posidonia*, its preferential habitat, from the Strait of Sicily and Western relatives (Serra et al., 2010) and can be related with the occurrence of rafting (Manly, 1976; Ávila, 2013). Gene flow and consequent maintenance of genetic similarity between Corsican and Italian populations benefit from the geographical proximity to the Ligurian Sea, with no known oceanographical discontinuities in the area (Fig. 6).

Notwithstanding the morphological resemblance of *T. tenuis* (Fig. 5G) and *T. pullus pullus* and the hypothesis of incomplete speciation proposed by Gofas (1982), our current dataset did not support a close phylogenetic relationship between these two taxa. *Tricolia tenuis* is reported as the only representative of the genus *Tricolia* in Cabo Verde (Rolán, 2005: fig. 143) but the distance, higher SSTs (~23–24 °C), and recent recognition of Cabo Verde as a separate biogeographic sub-province (Freitas et al., 2019) make it unlikely that it is the same species as in the Mediterranean Sea. It would be interesting to include Cabo Verdean specimens in future studies to determine the levels of differentiation of this insular taxon and assess its similarity to South African *Tricolia* species recently reviewed by Nangammbi et al. (2016), which greatly diverge from the Atlanto-Mediterranean *Tricolia* taxa (data not shown).

5. Conclusions

This work is the first phylogenetic study of the genus *Tricolia* in the NE Atlantic and Mediterranean Sea, substantially advancing the knowledge regarding its genetic and taxonomic diversity and complementing previous revisions based only on morphological characters. Our dataset comprised seven of the currently recognized *Tricolia* species, widely distributed in the study area. A “new” *Tricolia* sp. 1 was found to occur from Northern Portugal up to Brittany (France), although the clarification of its status (as a distinct species or just as a differentiated lineage) requires future studies, namely its morphological and ecological characterization. Molecular data revealed that only one species, *T. azorica* (Dautzenberg, 1889), occurs in the NE Atlantic Archipelagos (Azores, Madeira, Selvagens, and Canaries). Within the remainder of the *T. pullus* group (*sensu* Gofas 1982), the Mediterranean and the Atlantic forms should instead be distinguished as valid species and the binomens *T. pullus* (Linnaeus, 1758) and *T. picta* (da Costa, 1778), respectively, should be restored to species rank. *Tricolia speciosa*, *T. tenuis*, and *T. miniata* also occur in the Mediterranean Sea. *Tricolia miniata* seems to be a complex of species in the Mediterranean, with divergence levels of 7% within the Alboran Sea, reaching up to 15% across the populations studied. A wider geographic and taxonomic sampling remains necessary for the taxonomical characterization, recognition of potential cryptic species, and accurate evaluation of the status of *T. miniata* as a species complex.

The phylogenetic relationships seem to reflect the preferred substrates of each species reported by Gofas (1982). Among large sized *Tricolia* species, *T. tenuis* and *T. speciosa* are the only obligate phanerogram dwellers, but their phylogenetic relationships remain unclear and would benefit from the inclusion of additional taxa and/or markers. With the current dataset, the high support of the cluster formed by *T. miniata*, *T. azorica*, *Tricolia* sp. 1, *T. picta* (algal dwellers), and *T. pullus* (phanerogram and algal dweller) suggests that phanerogram dwelling might constitute a plesiomorphic condition among large sized *Tricolia* in the NE Atlantic and Mediterranean.

Less widespread *Tricolia* species were not included in this molecular study: *T. algoidea* (Pallary, 1920) and *T. petiti* (Craven, 1882) in the Atlantic, *T. tingitana* Gofas, 1982, *T. nordsiecki* (Talavera, 1978), *T. deschampsii* Gofas, 1993, *T. entomocheila* Gofas, 1993, *T. punctura*

Gofas, 1993, and *T. landinii* Bogi and Campani, 2007 in the Mediterranean. In light of the present findings, we urge for the need of a full integrative taxonomic revision of the genus *Tricolia* in the NE Atlantic and the Mediterranean, including the currently missing taxa, to draw a comprehensive phylogenetic framework for this genus.

Data availability

The genetic data underlying this article is available in the GenBank Nucleotide Database (accession numbers ON970419-ON970472, ON971203-ON971219, and ON974897-ON974959 for the *cox1*; ON988103-ON988140 for the 28S; ON997565-ON997587 for the *ITS2*). The corresponding GenBank accession numbers can be accessed found in Supplementary Table S1.

CRedit authorship contribution statement

Lara Baptista: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Giulia Fassio:** Resources, Investigation, Validation, Writing – review & editing. **Serge Gofas:** Resources, Validation, Visualization, Writing – review & editing. **Marco Oliverio:** Resources, Validation, Writing – review & editing, Funding acquisition. **Sérgio P. Ávila:** Resources, Validation, Writing – review & editing. **António M. Santos:** Conceptualization, Validation, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have attached the link to my data in the manuscript, uploaded in the Attach File step.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia, IP (grant number SFRH/BD/135918/2018 to L.B.; research contract IF/00465/2015 to S.P.A.). Work supported by National Funds through FCT-Fundação para a Ciência e a Tecnologia in the scope of the project UIDP/50027/2020 and by FEDER funds through the Operational Programme for Competitiveness Factors – COMPETE (projects UIDB/50027/2020, UID/BIA/50027/2013, POCI-01-0145-FEDER-006821); by regional funding through Direção Regional para a Ciência e Tecnologia (DRCT-M1.1.a/005/Funcionamento-C/2018; M3.3.B/ORG.R.C./005/2021, M3.3.B/ORG.R.C./008/2022/EDIÇÃO 1, M3.3.G/EXPEDIÇÕES CIENTÍFICAS/005/2022 and M3.3.G/EXPEDIÇÕES CIENTÍFICAS/004/2022 and M3.3.G/EXPEDIÇÕES CIENTÍFICAS/005/2022). This work was also supported by FEDER funds (85%) and by funds of the Regional Government of the Azores (15%) through Programa Operacional Açores 2020, in the scope of the projects “VRPROTO”: Virtual Reality PROTOtype: the geological history of “Pedra-que-pica”: ACORES-01-0145-FEDER-000078, “AZORESBIOPORTAL – PORBIOTA”: ACORES-01-0145-FEDER-000072, Portal da Biodiversidade dos Açores (M1.1.A/INFRAEST CIENT/001/2022), and M1.1.A/INFRAEST CIENT/A/001/2021 - Base de Dados da PaleoBiodiversidade da Macaronésia. Licenses CCIP 35/2019/DRCT and AMP 2018/014 for sampling in the Azores Archipelago were issued by the Regional Government of the Azores. MNHN specimens were collected during scientific expeditions under the auspices of the Our Planet Reviewed program (CORSICABENTHOS 1, CORSICABENTHOS 2, CORSICABENTHOS 3, GABES 2012-2013, BIZERTE).

We thank Esther Martín-González and the organizing committee of the 1st Workshop of Marine Paleontology and Littoral Geology in Canary

Islands (1° MPLG-F), which allowed sampling at Fuerteventura; Eva Cacabelos for providing samples of *Tricolia pullus canarica* from Madeira Island; Nicolas Puillandre and Dario Zuccon for the *cox1* sequences produced at the Muséum National D’Histoire Naturelle (MNHN, Paris, France) and Barbara Buge for the help with MNHN molecular vouchers; Carlos Melo for assembling the raw maps for Figs. 1 and 5; Livia Siniaglia for the pictures of *T. azorica*; Giacomo Chiappa for the help with lab work and BAU photos. The authors acknowledge the access to the collections of the Department of Biology of the University of the Azores (DBUA) and CIBIO-InBIO. We thank the reviewers for their comments that greatly improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107857>.

References

- ABGD web, 2010. Automatic Barcode Gap Discovery webserver. accessed November 2022. <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>.
- Akaike, H., 1973. Information Theory and an Extension of the Maximum Likelihood Principle, in: Proceedings of the 2nd International Symposium on Information Theory. Budapest: Akademiai Kiado, pp. 267–281.
- Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* 25, 4692–4693. <https://doi.org/10.1093/NAR/25.22.4692>.
- ALTER, 2010. Alignment Transformation Environment. accessed February 2022. <http://www.sing-group.org/ALTER/>.
- ASAP web, 2023. Assemble Species by Automatic Partitioning webserver. accessed November 2022. <https://bioinfo.mnhn.fr/abi/public/asap/>.
- Ávila, S.P., 2006. Oceanic islands, rafting, geographical range and bathymetry: a neglected relationship? *Occ Publ Irish Biogeogr Soc* 9, 22–39.
- Ávila, S.P., 2013. Unravelling the patterns and processes of evolution of marine life in oceanic islands: a global framework. In: Fernández-Palacios, J.M., de Nascimento, L., Hernández, J.C., Clemente, S., González, A., Díaz-González, J.P. (Eds.), *Climate Change Perspectives From the Atlantic: Past, Present and Future*. Universidad de La Laguna, Tenerife, pp. 95–125.
- Ávila, S.P., Melo, C., Berning, B., Nuno, S., Quartau, R., Rijdsdijk, K.F., Ramalho, R.S., Cordeiro, R., de Sá, N.C., Pimentel, A., Baptista, L., Medeiros, A., Gil, A., Johnson, M. E., 2019. Towards a ‘Sea-Level Sensitive’ dynamic model: impact of island ontogeny and glacio-eustasy on global patterns of marine island biogeography. *Biol Rev* 94, 1116–1142. <https://doi.org/10.1111/brv.12492>.
- Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. *Molecular Ecology*. <https://doi.org/10.1046/j.1365-294X.2003.02063.x>.
- Baptista, L., Santos, A.M., Melo, C.S., Rebelo, A.C., Madeira, P., Cordeiro, R., Botelho, A. Z., Hipólito, A., Pombo, J., Voelker, A.H.L., Ávila, S.P., 2021a. Untangling the origin of the newcomer *Phorcus sauciatius* (Mollusca: Gastropoda) in a remote Atlantic archipelago. *Mar Biol* 168. <https://doi.org/10.1007/s00227-020-03808-5>.
- Baptista, L., Meimberg, H., Ávila, S.P., Santos, A.M., Curto, M., 2021b. Dispersal ability, habitat characteristics, and sea-surface circulation shape population structure of *Cingula trifasciata* (Gastropoda: Rissoidae) in the remote Azores Archipelago. *BMC Ecol Evol* 21, 128. <https://doi.org/10.1186/s12862-021-01862-1>.
- Barco, A., Aissaoui, C., Houart, R., Bonomolo, G., Crocetta, F., Oliverio, M., 2018. Revision of the *Ocenebrina aciculata* species complex (Mollusca: Gastropoda: Muricidae) in the northeastern Atlantic Ocean and Mediterranean Sea. *J Molluscan Stud* 84, 19–29. <https://doi.org/10.1093/MOLLUS/EYX039>.
- Bogi, C., Campani, E., 2007. *Tricolia landinii*, una nuova specie per le coste orientali della Sicilia. *Iberus* 25, 27–31.
- Borges, L.M.S., Sivrikaya, H., le Roux, A., Shipway, J.R., Cragg, S.M., Costa, F.O., 2012. Investigating the taxonomy and systematics of marine wood borers (Bivalvia: Terebridae) combining evidence from morphology, DNA barcodes and nuclear locus sequences. *Invertebr Syst* 26, 572–582. <https://doi.org/10.1071/IS12028>.
- Borges, L.M.S., Hollatz, C., Lobo, J., Cunha, A.M., Vilela, A.P., Calado, G., Coelho, R., Costa, A.C., Ferreira, M.S.G., Costa, M.H., Costa, F.O., 2016. With a little help from DNA barcoding: Investigating the diversity of Gastropoda from the Portuguese coast. *Sci Rep* 6. <https://doi.org/10.1038/srep20226>.
- bPPT server, 2015. A Bayesian implementation of the PTP model for species delimitation. accessed April 2022. <https://mptp.h-its.org/#/tree>.
- Calvo, M., Templado, J., Oliverio, M., Machordom, A., 2009. Hidden Mediterranean biodiversity: molecular evidence for a cryptic species complex within the reef building vermetid gastropod *Dendropoma petraeum* (Mollusca: Caenogastropoda). *Biol J Linn Soc* 96, 898–912. <https://doi.org/10.1111/j.1095-8312.2008.01167.x>.
- Calvo, M., Alda, F., Oliverio, M., Templado, J., Machordom, A., 2015. Surviving the Messinian Salinity Crisis? Divergence patterns in the genus *Dendropoma* (Gastropoda: Vermetidae) in the Mediterranean Sea. *Mol Phyl Evol* 91, 17–26. <https://doi.org/10.1016/j.ympev.2015.05.004>.
- Castresana, J., 2000. Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Mol Biol Evol* 17, 540–552.

- Chen, H., Strand, M., Norenburg, J.L., Sun, S., Kajihara, H., Chernyshev, A.V., Maslakova, S.A., Sundberg, P., 2010. Statistical Parsimony Networks and Species Assemblages in Cephalotrichid Nemertean (Nemertea). *PLoS One* 5, e12885.
- Chernomor, O., von Haeseler, A., Minh, B.Q., 2016. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Syst Biol* 65. <https://doi.org/10.1093/sysbio/syw037>.
- Claremont, M., Williams, S.T., Barraclough, T.G., Reid, D.G., 2011. The geographic scale of speciation in a marine snail with high dispersal potential. *J Biogeogr* 38, 1016–1032. <https://doi.org/10.1111/j.1365-2699.2011.02482.x>.
- Clement, M., Posada, D., Crandall, K.A., 2000. TCS: A computer program to estimate gene genealogies. *Mol Ecol* 9, 1657–1659. <https://doi.org/10.1046/j.1365-294X.2000.01020.x>.
- Cordeiro, R., Borges, J.P., Martins, A.M.F., Ávila, S.P., 2015. Checklist of the littoral gastropods (Mollusca: Gastropoda) from the Archipelago of the Azores (NE Atlantic). *Biodivers J* 6, 855–900.
- Crocetta, F., Caputi, L., Paz-Sedano, S., Tanduo, V., Vazzana, A., Oliverio, M., 2020. High genetic connectivity in a gastropod with long-lived planktonic larvae. *J Moll Stud* 86 (1), 42–55.
- da Costa, E.M., 1778. *Historia naturalis testaceorum Britanniae*. Robson and White, London, Milan.
- Desiderato, A., Costa, F.O., Serejo, C.S., Abbiati, M., Queiroga, H., Vieira, P.E., 2019. Macaronesian islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae (Crustacea, Amphipoda). *Zool Scr* 48, 359–375. <https://doi.org/10.1111/ZSC.12339>.
- Després, L., 2019. One, two or more species? Mitonuclear discordance and species delimitation. *Mol Ecol* 28, 3845–3847. <https://doi.org/10.1111/MEC.15211>.
- Fassio, G., Modica, M.V., Alvaro, M.C., Buge, B., Salvi, D., Oliverio, M., Schiaparelli, S., 2019. An Antarctic flock under the Thorson's rule: Diversity and larval development of Antarctic Velutinidae (Mollusca: Gastropoda). *Mol Phyl Evol* 132, 1–13. <https://doi.org/10.1016/j.ympev.2018.11.017>.
- Fassio, G., Bouchet, P., Lozouet, P., Modica, M.V., Russini, V., Schiaparelli, S., Oliverio, M., 2021. Becoming a limpet: An “intermittent limpetization” process driven by host features in the kleptoparasitic gastropod family Capulidae. *Mol Phyl Evol* 155, 107014. <https://doi.org/10.1016/j.ympev.2020.107014>.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3, 294–299. <https://doi.org/10.1371/journal.pone.0013102>.
- Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J.A., Wirtz, P., Falcón, J.M., Brito, A., Floeter, S.R., Afonso, P., Porteiro, F., Viera-Rodríguez, M.A., Neto, A.I., Haroun, R., Farminhão, J.N.M., Rebelo, A.C., Baptista, L., Melo, C.S., Martínez, A., Núñez, J., Berning, B., Johnson, M.E., Ávila, S.P., 2019. Restructuring of the ‘Macaronesia’ biogeographic unit: A marine multi-taxon biogeographical approach. *Sci Rep* 9, 1–18. <https://doi.org/10.1038/s41598-019-51786-6>.
- Gamliel, I., Buba, Y., Guy-Haim, T., Garval, T., Willette, D., Rilov, G., Belmaker, J., 2020. Incorporating physiology into species distribution models moderates the projected impact of warming on selected Mediterranean marine species. *Ecography (Cop.)* 43, 1090–1106. <https://doi.org/10.1111/ECOG.04423>.
- GEBCO, 2020. Gridded Bathymetry Data. accessed March 2022. https://www.gebco.net/data_and_products/gridded_bathymetry_data/.
- Geller, J., Meyer, C., Parker, M., Hawk, H., 2013. Redesign of PCR primers for mitochondrial Cytochrome C Oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Mol Ecol Res* 13, 851–861. <https://doi.org/10.1111/1755-0998.12138>.
- GenBank, 2013. NIH genetic sequence database. accessed for sequence submission on August 2022. <https://www.ncbi.nlm.nih.gov/genbank/>.
- Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., Posada, D., 2010. ALTER: program-oriented format conversion of DNA and protein alignments. *Nucleic Acids Res. Web Server issue*. <https://doi.org/10.1093/nar/gkq321>.
- Gofas, S., 1982. The genus *Tricolia* in the Eastern Atlantic and the Mediterranean. *J Molluscan Stud* 48, 182–213.
- Gofas, S., 1986. Taxonomie des *Tricolia* Méditerranéennes. *Lavori della Società Italiana di Malacologia* 22, 179–184.
- Gofas, S., 1993. Notes on some Ibero-Moroccan and Mediterranean *Tricolia* (Gastropoda, Tricolidae), with descriptions of new species. *J Molluscan Stud* 59, 351–361. <https://doi.org/10.1093/mollus/59.3.351>.
- Gubbay, S., Sanders, N., Haynes, T., Janssen, J.A.M., Rodwell, J.R., Nieto, A., Garcia Criado, M., Beal, S., Borg, J., 2016. European Red List of Habitats Environment. Part 1: Marine habitats. *European Union*. 10.2779/032638.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* 59. 10.1093/sysbio/syq010.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41, 95–98.
- Hart, M.W., Sunday, J., 2007. Things fall apart: Biological species form unconnected parsimony networks. *Biol Lett* 3, 509–512. <https://doi.org/10.1098/rsbl.2007.0307>.
- Hassouna, N., Mithot, B., Bachelier, J.P., 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Res* 12, 3563–3583. <https://doi.org/10.1093/NAR/12.8.3563>.
- Hayward, P., Ryland, J.S., 2017. *Handbook of the Marine Fauna of North-West Europe*, 2nd ed. Oxford University Press.
- Hirahara, S., Ishii, M., Fukuda, Y., 2014. Centennial-Scale Sea Surface Temperature Analysis and Its Uncertainty. *J Clim* 27, 57–75. <https://doi.org/10.1175/JCLI-D-12-00837.1>.
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol Biol Evol* 35. <https://doi.org/10.1093/molbev/msx281>.
- Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., Flouri, T., 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* 33. <https://doi.org/10.1093/bioinformatics/btx025>.
- Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Bioinformatics* 20, 1160–1166. <https://doi.org/10.1093/bib/bbx108>.
- Portuguese Hydrographic Institute accessed December 2019 <https://www.hidrografico.pt/op/33>.
- Kuraku, S., Zmasek, C. M., Nishimura, O., Katoh, K., 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Res* 41, W22–W28. 10.1093/nar/gkt389.
- Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Mol Biol Evol* 34, 772–773. <https://doi.org/10.1093/molbev/msw260>.
- MAFFT v7 online server, 2019. Multiple alignment program for amino acid or nucleotide sequences. accessed February 2022. <https://mafft.cbrc.jp/alignment/server/>.
- Manly, R., 1976. The larval development of *Tricolia pullus* (L.). *J Molluscan Stud* 42, 361–369.
- Marzouk, Z., Aurelle, D., Said, K., Chenuil, A., 2017. Cryptic lineages and high population genetic structure in the exploited marine snail *Hexaplex trunculus* (Gastropoda: Muricidae). *Biol J Linn Soc* 122, 411–428. <https://doi.org/10.1093/biolinnean/blx070>.
- Minh, B.Q., Nguyen, M.A.T., Von Haeseler, A., 2013. Ultrafast Approximation for Phylogenetic Bootstrap. *Mol Biol Evol* 30, 1188–1195. <https://doi.org/10.1093/MOLBEV/MST024>.
- Modica, M.V., Russini, V., Fassio, G., Oliverio, M., 2017. Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Mar Environ Res* 127, 92–101. <https://doi.org/10.1016/j.marenvres.2017.04.001>.
- Monterosato, di T.A., 1889. *Coquilles marines marocaines*. *Journal de Conchyliologie* 31, 20–40.
- mPTP, 2017. Multi-rate Poisson Tree Process. accessed April 2022. <https://mptp.h-its.org/#/tree>.
- Muñoz, M., Reul, A., Plaza, F., Gómez-Moreno, M.L., Vargas-Yañez, M., Rodríguez, V., Rodríguez, J., 2015. Implication of regionalization and connectivity analysis for marine spatial planning and coastal management in the Gulf of Cadiz and Alboran Sea. *Ocean Coast Manag* 118, 60–74. <https://doi.org/10.1016/J.OCECOAMAN.2015.04.011>.
- Nangambi, T.C., Herbert, D.G., Teske, P.R., 2016. Molecular insights into species recognition within southern Africa's endemic *Tricolia* radiation (Vetigastropoda: Pectinellidae). *J Molluscan Stud* 82, 97–103. <https://doi.org/10.1093/mollus/eyv037>.
- Oliverio, M., Mariottini, P., 2001. Contrasting morphological and molecular variation in *Coralliophila meyerdorffii* (Muricidae, Coralliophiliinae). *J Molluscan Stud* 67, 243–246.
- Oliverio, M., Cervelli, M., Mariottini, P., 2002. ITS2 rRNA evolution and its congruence with the phylogeny of muricid neogastropods (Caenogastropoda, Muricoidea). *Mol Phylogenet Evol* 25, 63–69. [https://doi.org/10.1016/S1055-7903\(02\)00227-0](https://doi.org/10.1016/S1055-7903(02)00227-0).
- Pascual, M., River, B., Schunter, C., Macpherson, E., 2017. Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS One* 12. <https://doi.org/10.1371/journal.pone.0176419>.
- Patarnello, T., Volckaert, F.A.M.J., Castilho, R., 2007. Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break? *Mol Ecol* 16, 4426–4444. <https://doi.org/10.1111/j.1365-294X.2007.03477.x>.
- Pond, S.L.K., Poon, A.F.Y., Frost, S.D.W., 2009. Estimating selection pressures on alignments of coding sequences. In: Lemey, P., Salemi, M., Vandamme, A. (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis*. Cambridge University Press, pp. 419–451.
- Puillandre, N., Baylac, M., Boisselier, M., Cruaud, C., Samadi, S., 2009. An integrative approach to species delimitation in *Benthomangelia* (Mollusca : Conoidea). *Biol J Linn Soc* 96, 696–708. <https://doi.org/10.1111/j.1095-8312.2008.01143.x>.
- Puillandre, N., Meyer, C.P., Bouchet, P., Olivera, B.M., 2011. Genetic divergence and geographic variation in the deep-water *Conus orbigny* complex (Mollusca: Conoidea). *Zool Scr* 40, 350–363. <https://doi.org/10.1111/J.1463-6409.2011.00478.X>.
- Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012a. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* 21, 1864–1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x>.
- Puillandre, N., Modica, M.V., Zhang, Y., Sirovich, L., Boisselier, M., 2012b. Large-scale species delimitation method for hyperdiverse groups. *Mol Ecol* 21, 2671–2691. <https://doi.org/10.1111/j.1365-294X.2012.05559.x>.
- Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. *Mol. Ecol. Resour.* 21, 609–620. <https://doi.org/10.1111/1755-0998.13281>.
- Quinteiro, J., Rodríguez-Castro, J., López, P., López-Jurado, L.F., González-Henríquez, N., Rey-Méndez, M., 2011. Morphological and molecular characterization of a new species of Atlantic stalked barnacle (Scalpelliformes: Pollicipedidae) from the Cape Verde Islands. *J Mar Biol Assoc UK* 91, 1537–1545. <https://doi.org/10.1017/S0025315411000166>.
- Quinteiro, J., Rodríguez-Castro, J., Rey-Méndez, M., Henríquez, N.G., 2020. Phylogeography of the insular populations of common octopus, *Octopus vulgaris*

- Cuvier, 1797, in the Atlantic macaronesia. *PLoS ONE* 15. <https://doi.org/10.1371/journal.pone.0230294>.
- Ratnasingham, S., Hebert, P.D.N., 2007. Bold: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Mol Ecol Notes* 7, 355–364. <https://doi.org/10.1111/J.1471-8286.2007.01678.X>.
- Riesgo, A., Taboada, S., Pérez-Portela, R., Melis, P., Xavier, J.R., Blasco, G., López-Legentil, S., 2019. Genetic diversity, connectivity and gene flow along the distribution of the emblematic Atlanto-Mediterranean sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *BMC Evol Biol* 19. <https://doi.org/10.1186/S12862-018-1343-6>.
- Rolán, E., 2005. *Malacological Fauna from the Cape Verde Archipelago*. ConchBooks, Hackenheim, Germany, p. 455.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61, 539–542. <https://doi.org/10.1093/sysbio/sys029>.
- Salemi, M., 2009. Genetic distances and nucleotide substitution models. In: Lemey, P., Salemi, M., Vandamme, A. (Eds.), *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis*. Cambridge University Press, pp. 126–140.
- Sá-Pinto, A., Branco, M., Sayanda, D., Alexandrino, P., 2008. Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the Macaronesian Islands. *Mol Ecol* 17, 519–532. <https://doi.org/10.1111/J.1365-294X.2007.03563.X>.
- Sá-Pinto, A., Baird, S.J.E., Pinho, C., Alexandrino, P., Branco, M., 2010. A three-way contact zone between forms of *Patella rustica* (Mollusca: Patellidae) in the central Mediterranean Sea. *Biol J Linn Soc* 100, 154–169. <https://doi.org/10.1111/j.1095-8312.2010.01405.x>.
- Sá-Pinto, A., Branco, M.S., Alexandrino, P.B., Fontaine, M.C., Baird, S.J.E., 2012. Barriers to Gene Flow in the Marine Environment: Insights from Two Common Intertidal Limpet Species of the Atlantic and Mediterranean. *PLOS One* 7, e50330.
- Scheltema, R.S., 1986. On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bull Mar Sci* 39, 290–322.
- Scheltema, R.S., 1989. Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In: Ryland, J.S., Tyles, P.A. (Eds.), *Reproduction, Genetics and Distribution of Marine Organisms*. Fredensborg, Olsen and Olsen, pp. 183–188.
- Scheltema, R.S., 1995. The relevance of passive dispersal for the biogeography of Caribbean mollusks. *Am Malacol Bull* 11, 99–115.
- Scuderi, D., Reitano, A., 2012. New observations on the taxonomy, biology and distribution of *Tricolia landinii* Bogi et Campani, 2007 (Gastropoda Vetigastropoda). *Biodivers J*.
- Clustal Omega, 2011. Multiple sequence alignment at EMBL-EBI Web Services accessed February 2022 <https://www.ebi.ac.uk/Tools/msa/clustalo/>.
- NOAA/OAR/ESRL PSL. NOAA Physical Sciences Laboratory accessed May 2022 <https://psl.noaa.gov/data/gridded/data.cobe2.html>.
- Santos, A.M., Cabezas, M.P., Tavares, A.I., Xavier, R., Branco, M., 2016. tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics* 32, 1019/3. <https://doi.org/10.1093/bioinformatics/btv636>.
- Segers, W., Swinnen, F., Abreu, A., 2009. An annotated checklist of the marine molluscs from the Archipelago of Madeira and the Selvagens (NE Atlantic Ocean). *Bocagiana* (Funchal) 226.
- Serra, I.A., Innocenti, A.M., di Maida, G., Calvo, S., Migliaccio, M., Zambianchi, E., Pizzigalli, C., Arnaud-Haond, S., Duarte, C.M., Serrão, E.A., Procaccini, G., 2010. Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. *Mol Ecol* 19, 557–568. <https://doi.org/10.1111/J.1365-294X.2009.04462.X>.
- Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7. <https://doi.org/10.1038/MSB.2011.75>.
- Smriglio, C., Furfaro, G., Trillò, P., Appolloni, M., Mariottini, P., 2019. A review of the Atlantic-Mediterranean *Bursa scrobilator* (Linnaeus, 1758) species complex. *Molluscan Res* 39, 341–354. <https://doi.org/10.1080/13235818.2019.1600397>.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. *Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas*. *BioScience* 57, 573–583. <https://doi.org/10.1641/B570707>.
- Talavera, G., Castresana, J., 2007. Improvement of Phylogenies after Removing Divergent and Ambiguously Aligned Blocks from Protein Sequence Alignments. *Syst Biol* 56, 564–577. <https://doi.org/10.1080/10635150701472164>.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol* 38. <https://doi.org/10.1093/molbev/msab120>.
- BOLD Systems v3, 2014. The Barcode of Life Data System first accessed December 2020, last accessed May 2022. <https://v3.boldsystems.org/>.
- Telesca, L., Belluscio, A., Criscoli, A., Ardizzone, G., Apostolaki, E.T., Fraschetti, S., Gristina, M., Knittweis, L., Martin, C.S., Pergent, G., Alagna, A., Badalamenti, F., Garofalo, G., Gerakaris, V., Louise Pace, M., Pergent-Martini, C., Salomidi, M., 2015. Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. *Sci Rep* 2015 5:1 5, 1–14. [10.1038/srep12505](https://doi.org/10.1038/srep12505).
- Thiel, M., Gutow, L., 2005a. The ecology of rafting in the marine environment. I. The floating substrata. *Oceanography and Marine Biology: An Annual Review*. <https://doi.org/10.1201/9780203507810>.
- Thiel, M., Gutow, L., 2005b. The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology* 43, 279–418. <https://doi.org/10.1201/9781420037449.ch7>.
- Thiel, M., Haye, P.A., 2006. The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanography and Marine Biology* 44, 323–429.
- Trifinopoulos, J., Nguyen, L.T., von Haeseler, A., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44. <https://doi.org/10.1093/NAR/GKW256>.
- Urra, J., Mateo Ramírez, Á., Marina, P., Salas, C., Gofas, S., Rueda, J.L., 2013. Highly diverse molluscan assemblages of *Posidonia oceanica* meadows in northwestern Alboran Sea (W Mediterranean): Seasonal dynamics and environmental drivers. *Estuar Coast Shelf Sci* 117, 136–147. <https://doi.org/10.1016/J.ECSS.2012.11.005>.
- Vieira, P.E., Desiderato, A., Holdich, D.M., Soares, P., Creer, S., Carvalho, G.R., Costa, F.O., Queiroga, H., 2019. Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates. *Mol Ecol* 28, 1784–1800. <https://doi.org/10.1111/mec.15052>.
- Vieira, P.E., Desiderato, A., Azevedo, S.L., Esquete, P., Costa, F.O., Queiroga, H., 2022. Molecular evidence for extensive discontinuity between peracarid (Crustacea) fauna of Macaronesian islands and nearby continental coasts: over fifty candidate endemic species. *Mar Biol* 169, 1–13. <https://doi.org/10.1007/S00227-022-04051-W/FIGURES/3>.
- ExpAsy Translate Tool accessed January 2022 <https://web.expasy.org/translate/>.
- Wigham, G.D., Graham, A., 2017. Marine gastropods. 1, Patellostropoda and Vetigastropoda, in: *Synopses of the British Fauna (New Series)*, No. 60. The Linnean Society of London, p. 172.
- Wilke, T., Davis, G.M., 2000. Intraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? *Biol J Linn Soc* 70, 89–105. <https://doi.org/10.1006/bjil.1999.0388>.
- Williams, S.T., Ozawa, T., 2006. Molecular phylogeny suggests polyphyly of both the turban shells (family Turbinidae) and the superfamily Trochoidea (Mollusca: Vetigastropoda). *Mol Phylogenet Evol* 39, 33–51. <https://doi.org/10.1016/J.YMPEV.2005.12.017>.
- Williams, S.T., Karube, S., Ozawa, T., 2008. Molecular systematics of Vetigastropoda: Trochidae, Turbinidae and Trochoidea redefined. *Zool Scr* 37, 483–506. <https://doi.org/10.1111/J.1463-6409.2008.00341.X>.
- Winston, J.E., 2012. Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*. 447–457. <https://doi.org/10.1093/icb/ics040>.
- W-IQ-Tree, 2016. IQ-TREE web server: fast and accurate phylogenetic trees under maximum likelihood. accessed May 2022. <http://iqtree.cibiv.univie.ac.at/>.
- Yeates, D.K., Seago, A., Nelson, L., Cameron, S.L., Joseph, L.E.O., Trueman, J.W., 2011. Integrative taxonomy, or iterative taxonomy? *Syst Entomol* 36 (2), 209–217. <https://doi.org/10.1111/j.1365-3113.2010.00558.x>.
- Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29. <https://doi.org/10.1093/bioinformatics/btt499>.