

Reassessing North Eastern Atlantic-Mediterranean species of *Trapania* (Mollusca, Nudibranchia)

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Abstract

Trapania is the second largest genus belonging to the family Goniadorididae, of which most of the species are reported from Indo-Pacific waters. To date, there are nine species of *Trapania* distributed along the temperate coasts of the East Atlantic Ocean and Mediterranean Sea: *Trapania fusca*, *Trapania graeffei*, *Trapania hispalensis*, *Trapania lineata*, *Trapania maculata*, *Trapania ortei*, *Trapania pallida*, *Trapania sanctipetrensis* and *Trapania tartanella*. However, the validity of some of these species has been problematic due to uncertain taxonomic characteristics used for the differentiation of the species. The genus *Trapania* has a very uniformly external morphology and very similar internal anatomy. As a consequence, the features most commonly used to differentiate species have been the colour pattern of the body and the morphology of the radula. In the present study, we perform a morphological and molecular revision of the East Atlantic-Mediterranean species of the genus *Trapania*. Morphological analyses include dissections and scanning electron microscope photographs of radulae, labial cuticles and penises. Molecular work includes phylogenetic, species delimitation and haplotype network analyses. Our results bring doubt on the taxonomic characteristics used so far, suggesting that the richness of the North Eastern Atlantic-Mediterranean species has been overestimated. *Trapania hispalensis*, *T. lineata* and *T. pallida* are shown to belong to the same taxa, with *Trapania lineata* as senior synonym.

KEYWORDS

Goniadorididae, morphotypes, synonymy, systematics

1 | INTRODUCTION

The genus *Trapania* Pruvot-Fol, 1931, currently includes 47 species worldwide, being the second largest genus of the family Goniadorididae (MolluscaBase, 2022). The genus was erected in 1931 by Pruvot-Fol, assigning

Drepania fusca Lafont, 1874 as type species. Currently, more than half of the *Trapania* species are described in the contributions by Rudman (1987) and Gosliner and Fahey (2008), focused on species from the Indo-Pacific Ocean and, therefore, where the highest species richness is concentrated. The monophyly of the genus is supported

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by phylogenetic analyses based on morphological data (Gosliner & Fahey, 2008). *Trapania* is characterized by having a reduced mantle margin, with a single pair of curved extra-rhinophoral and extra-branchial appendages. The rhinophores are lamellate, and there are three tripinnate gill branches forming a semicircle around the anus. The radular formula is $N \times 1.0.1$, and the jaws have elements. The penis is armed (Gosliner & Fahey, 2008; Kress, 1968; Paz-Sedano et al., 2021; Rudman, 1987). The species of *Trapania* show a uniform body plan, with external morphology and internal features, such as the reproductive system, being very similar between species. Therefore, the main characteristics used to differentiate species have been the radula and the colour of the body (Cervera et al., 2000; Edmunds, 2009; Gosliner & Fahey, 2008; Moro & Ortea, 2015; Rudman, 1987).

However, in some cases, the intraspecific variability and interspecific similarities of these characteristics have also been questioned. Some species have very similar radulae, such as *Trapania graeffei* (Bergh, 1880) and *Trapania tartanella* (Kress, 1968) or *Trapania squama* Gosliner & Fahey, 2008, *Trapania toddi* Rudman, 1987, *Trapania melaina* Gosliner & Fahey, 2008 and *Trapania euryeia* Gosliner & Fahey, 2008 (Gosliner & Fahey, 2008). In contrast, intraspecific variability has been reported between different specimens of the same species, or even between different teeth within the same radula, as example in the species *Trapania maringa* Er. Marcus, 1957 or *Trapania maculata* Haefelfinger, 1960 (Cervera & García-Gómez, 1989a; Haefelfinger, 1960; Marcus, 1957; Templado et al., 1988). These variations could question the validity of the shape of the radula as a taxonomic characteristic to difference species, leaving the colour pattern of the species as the main useful feature. However, the variability in the colour of European species has also been questioned by some authors, waiting for future studies to clarify the validity or synonymy of some species (Edmunds, 2009; Rudman, 1987; Templado et al., 1988).

To date, there are 14 species of *Trapania* that inhabit the Atlantic Ocean. Four of these species are distributed in the West Atlantic (*T. maringa*, *T. dalva* Ev. Marcus, 1972, *Trapania bornellenae* Valdés, 2009 and *Trapania rocheae* Cetra & Roche, 2019), three in the subtropical East Atlantic (*T. luquei* Ortea, 1989, *T. canaria* Ortea & Moro, 2009 and *T. bajamarensis* Moro & Ortea, 2015) and seven bordering the temperate coasts of the East Atlantic (*T. fusca*, *T. maculata*, *T. pallida* Kress, 1968, *T. tartanella*, *T. hispalensis* Cervera & García-Gómez, 1989, *T. oratei* García-Gómez & Cervera in Cervera & García-Gómez, 1989 and *T. sanctipetrensis* Cervera et al., 2000). Amongst these last seven species, *T. maculata*, *T. pallida*, *T. tartanella*, *T. hispalensis* and *T. oratei* also entered the Mediterranean Sea, inhabiting the basin together with *T. lineata* Haefelfinger, 1960 and *T. graeffei* (Cattaneo-Vietti & Thompson, 1989;

Cervera & García-Gómez, 1989a; Cervera et al., 2000; Haefelfinger, 1960; Just & Edmunds, 1985; Kress, 1968; Trainito & Doneddu, 2014; Trainito et al., 2018). However, the biodiversity of the *Trapania* species that inhabit the East Atlantic-Mediterranean area has been problematic, with questions regarding the validity or synonymy of some species. In 1954, Pruvot-Fol considered the species *T. graeffei* and *T. tartanella* as synonyms of *T. fusca*. Afterwards, most authors considered *T. tartanella* as a valid, distinct species (Cattaneo-Vietti & Thompson, 1989; Cervera et al., 2000; Haefelfinger, 1960; Ortea et al., 1989). In addition, the striking similarity in the colour pattern of *T. tartanella* and *T. hispalensis* was noted (Cervera & García-Gómez, 1989b; Doneddu et al., 2020), but they were never proposed as synonyms. Regarding *T. graeffei*, several authors agreed with the synonymy, and therefore, the species *T. fusca* was reported by different authors as being widely distributed from the Atlantic coast of France to the Mediterranean coasts of France, Italy and Malta (Kress, 1970; Nordsieck, 1972; Pruvot-Fol, 1954; Rudman, 1987; Sabelli et al., 1990; Sammut & Perrone, 1998; Schmekel & Portmann, 1982). However, at the same time, other authors referred to these species as distinct valid taxa (Cervera et al., 2000; Haefelfinger, 1960). Because of this disagreement, the validity, characteristics and distribution of this, or these, species remained uncertain. Recently, Doneddu et al. (2020) revised all the different publications including those with *T. fusca* and *T. graeffei* and concluded that both are valid species until proven otherwise, limiting the distribution of the species *T. fusca* to the type locality (Arcachon, Atlantic Coast of France) and assigning to *Trapania graeffei* the remaining reports along the Mediterranean Sea. These changes were mainly due to the lack of information in the original descriptions of the species.

In order to clarify the biodiversity of the North Eastern Atlantic-Mediterranean species of *Trapania*, and reviewing the characteristics used to differentiate them, specimens of eight of the nine species that inhabit the area (according to their previous identifications) were studied. Morphological analyses were performed by dissections. We included scanning electron microscope photographs of the radulae, labial cuticles and penial spines. Moreover, partial sequences of mitochondrial and nuclear genes were obtained, and a phylogenetic species delimitation and haplotype network analyses were carried out.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

Between 2008 and 2021, 55 specimens were collected by SCUBA diving along the coast of Ireland, France, Spain,

Morocco and Italy (Table S1). Also, the type material of some species was loaned by different Museums (Table S1). The newly collected specimens matched with eight morphospecies according to their initial identifications, including *Trapania* cf. *fusca*, *Trapania hispalensis*, *Trapania lineata*, *Trapania maculata*, *Trapania ortei*, *Trapania pallida*, *Trapania sanctipetrensis* and *Trapania tartanella*. Most of the specimens were preserved in 96% ethanol, and some of them were preserved in formalin. Specimens examined were deposited at the Museo Nacional de Ciencias Naturales, Madrid (Spain). Holotypes of *T. lineata*, *T. maculata* and *T. pallida*, preserved in Bouin's fixative, were loaned by the Natural History Museum Basel (Switzerland). The neotype of *T. tartanella*, preserved in 4% formalin, was loaned by the Museo Nacional de Ciencias Naturales, Madrid (Spain) (Table S1). Initial sampling for molecular analyses included a total of 21 specimens. In addition, 15 taxa were added from GenBank, seven belonging to the family Goniadorididae, seven species belonging to the superfamily Onchidoridoidea, and the pleurobranch *Berthella martensi* (Pilsbry, 1896) was used as outgroup (Table 1).

2.2 | Molecular analysis. DNA extraction, amplification and sequencing

DNA was extracted from a small sample of foot tissue using DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. Partial sequences of cytochrome oxidase c subunit I (COI), 16S ribosomal RNA (16S) and Histone H3 (H3) were amplified by polymerase chain reaction (PCR) using universal primers LCO1490 and HCO2198 for COI (Folmer et al., 1994), 16Sar-L and 16Sbr-H for 16S (Palumbi, 1996), and H3AD5'3' and H3BD5'3' primers for H3 (Colgan et al., 1998). Final volume reaction of each PCR was 25 μ l, including: 2.5 μ l of CoralLoad PCR Buffer, 2.5 μ l of dNTP (2 mM), 3.5 μ l of MgCl₂ (25 mM) for COI and 16S, 2 μ l of MgCl₂ (25 mM) for H3, 0.25 μ l of Qiagen DNA polymerase (5 μ l/ μ l), 1 μ l of each forward and reverse primer, 5 μ l of 'Q-Solution' and 2 μ l of DNA and 7.25 μ l and 8.75 μ l of MiliQ water for COI and 16S, and H3, respectively. COI amplification was performed with an initial denaturation of 3 min at 95.0°C, followed by 40 cycles of 45 s at 94.0°C, 45 s at 45.0°C (annealing temperature) and 2 min at 72.0°C with a final extension of 10 min at 72°C. Amplification of 16S was performed with an annealing temperature of 51.5°C, and 50°C for H3. Successful PCR products obtained were sequenced in Macrogen, Inc. All new sequences obtained were deposited in GenBank (Table 1).

2.3 | Phylogenetic analyses

Successful sequences were assembled and edited using SeqMan II software (DNASTar, Madison, WI, USA). The alignment was done using MEGA7 (Kumar et al., 2016). Protein-coding sequences were translated into amino acids for confirmation of alignment using the genetic code invertebrate mitochondrial DNA for COI and universal code for H3. All sequences were blasted in GenBank to check for contamination. Sequences were trimmed to 658, 449 and 328 pair bases for COI, 16S and H3, respectively. The evolutionary models were selected using jModelTest2 on XEDE (2.1.6) available at CIPRES Science Gateway (Miller et al., 2010), for 16S gene and for each codon position of COI and H3, under Bayesian information criteria (BIC) (Schwarz, 1978). Evolutionary models for COI were TrN+G, TPM1uf+I and HKY+G for the first, second and third codon position, respectively. The TPM3uf+G evolutionary model was selected for 16S. For H3 gene, TIM2+I, JC and TPM2uf+G were selected for the first, second and third codon position, respectively. Bayesian inference (BI) analysis was performed using the software package MrBayes on XSEDE (3.2.7a), available at CIPRES Science Gateway (Miller et al., 2010), for ten million generations, four independent runs and sampling frequency of 1000. Nodes were considered supported by posterior probabilities ≥ 0.96 (Alfaro et al., 2003). Maximum likelihood (ML) analysis was performed using the software package RAXML-NG (Kozlov et al., 2019), with a bootstrapping cut-off of 0.03 implemented. Nodes were considered statistically significant by bootstraps values ≥ 75 (Hillis & Bull, 1993). Phylogenetic trees were conducted for individual genes and for concatenate of a minimum of two (COI+16S) (COI+H3) to three genes (COI+H3+16S). The tree obtained was shown using FigTree (Rambaut, 2009). To obtain the final tree, the results were edited in Adobe Photoshop CC 2014. Nodes with posterior probabilities and bootstraps values not supported were collapsed.

2.4 | Species delimitation analyses

Pairwise uncorrected *p*-distances for COI, 16S and H3 using MEGA7 (Kumar et al., 2016) were calculated for comparing the genetic distances amongst specimens of *Trapania*. Moreover, species delimitation analyses were conducted on COI and 16S. Bayesian Poisson Tree Process (bPTP) (Zhang et al., 2013) delimitation analysis was conducted using webtool (<https://species.h-its.org>), using the tree obtained in the Bayesian analyses as input, running 200,000 MCMC generations, Thinning = 100 and Burn-in = 0.1. Automatic Barcode Gap Discovery (ABGD)

TABLE 1 Species used for molecular analyses, initial identification of species, voucher, locality, and GenBank accession number

| Species | Initial identification | Voucher | Locality | COI | 16S | H3 |
|------------------------------------|-----------------------------|-------------------|--|----------|----------|----------|
| <i>Adalaria proxima</i> | - | CASIZ 183921A | USA, Maine, Washington, Passamaquoddy Bay Eastport | KM219676 | KJ653673 | KM225826 |
| <i>Ancula gibbosa</i> | - | CASIZ 182028 | USA, Maine, Cumberland | KP340388 | KP340291 | KP340413 |
| <i>Atalodoris jannae</i> | - | CASIZ 175578 | USA, California, San Mateo, Pillar Point | KP340392 | KP340296 | KP340415 |
| <i>Armodoris anudeorum</i> | - | LACM 3118 | Antarctica, Ross Sea, McMurdo Sound, | KP340387 | KP340290 | KP340412 |
| <i>Berthella martensi</i> | - | MZUCR 6982 | Panama, Las Secas | HM162683 | HM162592 | HM162498 |
| <i>Corambe obscura</i> | - | CASIZ 183942 | USA, New Hampshire, Rockingha, New Castle Portsmouth Bay | KP340399 | KP340303 | KP340419 |
| <i>Corambe pacifica</i> | - | LACM 2007-2.6B | USA, California, Los Angeles, Long Beach Marina | KP340400 | KP340304 | KP340420 |
| <i>Diaphorodoris lirulatocauda</i> | - | CASIZ 184341 | USA, California, Duxbury Reef, Marin Co | KP340403 | KP340307 | KP340422 |
| <i>Diaphorodoris luteocincta</i> | - | LACM 8.7A | Spain, Cádiz, Bahía de Algeciras | KP340404 | KP340308 | KP340423 |
| <i>Goniodoris nodosa</i> | - | - | Sweden, Bohuslan, Kristineberg | AJ223264 | AJ225188 | - |
| <i>Okenia aurorapapillata</i> | - | MNHNC/MB28-004930 | Mozambique, Inhambane, Zavora | MW357570 | MW364867 | MW368320 |
| <i>Okenia elegans</i> | - | MNCN 15.05/88175 | Spain, La Planassa | MK645759 | MK650421 | MK659667 |
| <i>Okenia mediterranea</i> | - | MNCN 15.05/88174 | Italy, Santa Maria al Bagno | MK645760 | MK650422 | MK659668 |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MNCN 15.05/94359 | Morocco, Capghir | OM220097 | OM237318 | - |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MNCN 15.05/94360 | Morocco, Capghir | OM220098 | OM237319 | - |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MNCN 15.05/94366 | Morocco, Jebha | OM220099 | OM237320 | OM257117 |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MNCN 15.05/94370 | France, Cap Ferret, Hortense | OM220100 | OM237321 | OM257118 |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MNCN 15.05/94368 | Spain, Huelva, Cristina Island, Carreras River | OM220101 | OM237322 | OM257119 |
| <i>Trapania lineata</i> | <i>Trapania hispalensis</i> | MZCN 15.05/55504 | Portugal, Aveiro | JX274080 | JX274048 | - |
| <i>Trapania lineata</i> | <i>Trapania lineata</i> | MNCN 15.05/94345 | Italy, Naples | OM220102 | OM237323 | OM257120 |
| <i>Trapania lineata</i> | <i>Trapania lineata</i> | MNCN 15.05/94352 | Spain, Girona, Es Caials, Cadaqués | OM220103 | OM237324 | OM257121 |
| <i>Trapania lineata</i> | <i>Trapania lineata</i> | MNCN 15.05/94351 | Spain, Girona, Punta del romaní, L'Escala | OM220104 | OM237325 | - |
| <i>Trapania lineata</i> | <i>Trapania lineata</i> | MNCN 15.05/94353 | Spain, Menorca, Porros Island | OM220105 | OM237326 | OM257122 |
| <i>Trapania lineata</i> | <i>Trapania pallida</i> | MNCN 15.05/94371 | Ireland, Galway | OM220106 | OM237327 | - |
| <i>Trapania lineata</i> | <i>Trapania pallida</i> | MNCN 15.05/94372 | Ireland, Galway | OM220107 | OM237328 | - |
| <i>Trapania maculata</i> | <i>Trapania maculata</i> | MNCN 15.05/94373 | Spain, Girona, Punta del romaní, L'Escala | OM220108 | OM237329 | OM257123 |
| <i>Trapania maculata</i> | <i>Trapania maculata</i> | MNCN 15.05/94374 | Spain, Girona, Punta del romaní, L'Escala | OM220109 | OM237330 | OM257124 |
| <i>Trapania maculata</i> | <i>Trapania maculata</i> | MNCN 15.05/94375 | Spain, Girona, Punta del romaní, L'Escala | OM220111 | OM237331 | OM257125 |
| <i>Trapania ortei</i> | <i>Trapania ortei</i> | MNCN 15.05/94384 | Morocco, Capghir | OM220110 | OM237332 | OM257126 |
| <i>Trapania ortei</i> | <i>Trapania ortei</i> | MNCN 15.05/94389 | Morocco, Taghazout | OM220112 | OM237333 | OM257127 |

TABLE 1 (Continued)

| Species | Initial identification | Voucher | Locality | COI | 16S | H3 |
|---------------------------------|---------------------------------|------------------|---|----------|----------|----------|
| <i>Trapania ortei</i> | <i>Trapania ortei</i> | MNCN 15.05/94381 | Spain, Huelva, Ayamonte, Punta del Moral | OM220113 | OM237334 | OM257128 |
| <i>Trapania ortei</i> | <i>Trapania ortei</i> | MNCN 15.05/94383 | Spain, Huelva, Lepe, El Terrón, Arroyo del Fraile | OM220114 | OM237335 | OM257129 |
| <i>Trapania ortei</i> | <i>Trapania ortei</i> | MNCN 15.05/94382 | Spain, Huelva, Ayamonte, Punta del Moral | - | - | OM257130 |
| <i>Trapania reticulata</i> | - | CASIZ 191431 | Papua New Guinea, Tab Island. | MF958432 | MF958303 | - |
| <i>Trapania sanctipetrensis</i> | <i>Trapania cf. fusca</i> | MNCN 15.05/94390 | Spain, Huelva, Ayamonte, Punta del Moral | - | OM237316 | OM257115 |
| <i>Trapania sanctipetrensis</i> | <i>Trapania sanctipetrensis</i> | MNCN 15.05/94391 | Spain, Cádiz, Sancti Petri | - | OM237317 | OM257116 |

Note: Species newly sequenced during the present study are highlighted in bold.

Institutional acronyms: CASIZ, California Academy of Science; LACM, Los Angeles County Museum; MNCN, Museo Nacional de Ciencias Naturales; MNHNC/MB, Museu Nacional de Història Natural e da Ciência of Lisbon; MZUCR, Museo de Zoología Universidad de Costa Rica.

(Puillandre et al., 2012) was run using Kimura (K80) evolutionary model, with a relative gap width (X) of 1, a divergence of intraspecific diversity between 0.0001 and 0.1 and Nb bins = 20, using the webtool (<http://www.abi.snv.jussieu.fr/public/abgd/abgdweb.html>). Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021) were also conducted using the webtool (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>), under the model Kimura (K80) ts/tv.

2.5 | Haplotype network analysis

Haplotype network analysis was conducted for *Trapania lineata*, using the program PopArt v. 1.7 (Leigh & Bryant, 2015). Empty positions at both ends of the alignment were removed, leaving a final alignment with 565 bp of 12 taxa for COI. Trait file with geographic area codes was created with excel, assigning a value of 0 when specimens were absent and 1 when specimens were present. Alignment and trait files were imported to PopArt, and the TCS algorithm analysis (Clement et al., 2000) was conducted. Mutations are shown as one-step edges. The haplotype network was edited using PopArt v.1.7 and Adobe Photoshop CC 2014.

2.6 | Morphological examination

The external morphology was examined from photographs of living specimens and laboratory observations. Specimens were fully dissected for detailed study, and the digestive and reproductive systems were drawn under a Nikon SMZ-1500 dissecting microscope with a camera lucida attached. The buccal bulb was submerged in 10% NaOH to dissolve the tissue and musculature surrounding the radula and labial cuticle. Then, these structures were risen in distilled water. Penises and labial cuticles were critically dried using hexamethyldisilazane, and all structures were examined under a Hitachi S3000N scanning electron microscope (SEM) at 'Servicio Interdepartamental de Investigación', Autonomous University of Madrid, Spain.

3 | RESULTS

3.1 | Phylogenetic and species delimitation analyses

We successfully obtained sequences for the 21 taxa initially sampled (Table 1). The highest intraspecific distance between two specimens of same species were 2.1% for COI, 1.3% for 16S and 0.5% for H3 (Table 2). In addition, the lowest values obtained between two different

| Species | COI | 16S | H3 |
|--|-----------|---------|---------|
| Within <i>Trapania maculata</i> | 0.4 | 0–0.3 | 0 |
| Within <i>Trapania ortei</i> | 0–2.1 | 0–0.3 | 0–0.5 |
| Within <i>Trapania lineata</i> | 0–2.1 | 0–1.3 | 0 |
| Within <i>Trapania sanctipetrensis</i> | – | 0 | 0 |
| <i>Trapania maculata</i> vs. <i>Trapania ortei</i> | 13.5–14.6 | 3.8–4.1 | 6.4–7.0 |
| <i>Trapania maculata</i> vs. <i>Trapania lineata</i> | 9.8–10.9 | 4.1–4.4 | 5.3 |
| <i>Trapania maculata</i> vs. <i>Trapania sanctipetrensis</i> | – | 5.0–5.4 | 6.4 |
| <i>Trapania ortei</i> vs. <i>Trapania lineata</i> | 10.9–11.9 | 3.8–4.4 | 2.7–3.2 |
| <i>Trapania ortei</i> vs. <i>Trapania sanctipetrensis</i> | – | 3.8 | 4.3–4.8 |
| <i>Trapania lineata</i> vs. <i>Trapania sanctipetrensis</i> | – | 4.4–4.7 | 2.7 |

TABLE 2 COI, 16S and H3 gene pairwise uncorrected *p*-distances (%) within and amongst species of *Trapania*

species were 9.8% for COI, 3.8% for 16S and 2.7% for H3. Uncorrected *p*-distances, ASAP and ABGD species delimitation analyses performed in COI and 16S, and bPTP performed in 16S, showed two remarkable results: (i) the specimen initially identified as *Trapania cf. fusca* (MNCN 15.05/94390) from Huelva (Spain) and the specimen of *Trapania sanctipetrensis* (MNCN 15.05/94391) from Cádiz (Spain) belonged to the same taxa, with a *p*-distance of 0% in both 16S and H3 genes (Table 2; Figure 1), henceforth referred as *Trapania sanctipetrensis*; (ii) specimens of *Trapania lineata*, *Trapania pallida* and *Trapania hispalensis* were considered the same species, with a *p*-distance of 2.1%, 1.3% and 0% in COI, 16S and H3 genes, respectively (Table 2; Figure 1), supporting the synonymy of *T. pallida* and *T. hispalensis* with *T. lineata*. *Trapania maculata* as well as *Trapania ortei* were confirmed as valid species (Figure 1). On the contrary, bPTP species delimitation analysis performed in COI divided the specimens of *T. ortei* and *T. lineata* in different taxa. Specimens of *T. ortei* from Morocco were considered different taxa from those collected in Spain (maximum COI *p*-distances 2.1%). Regarding *T. lineata*, bPTP analysis divided the species in five taxa. Two of these taxa included morphotypes of *T. lineata* and the synonymized *T. hispalensis*. The two specimens of the morphotype corresponding to the synonymized *T. pallida* were considered one different taxon.

The phylogenetic tree obtained based on concatenate gene sequences supported the monophyly of the genus by grouping *Trapania* taxa into a well-supported clade (BI = 1; ML = 100) (Figure 1). Although only five species of *Trapania* were included, some relationships between species were well-supported by Bayesian Inference analysis. The first clade gathered the East Atlantic-Mediterranean *Trapania* species, separated from *T. reticulata* Rudman, 1987 (BI = 0.98). Amongst them, *T. sanctipetrensis* and *T. ortei* joined as sister species (BI = 0.99), and these two species clustered with *T. lineata* (BI = 1).

3.2 | Haplotype network analysis

The haplotype network included the 12 specimens of *Trapania lineata*, grouped as the same taxa in ABGD and ASAP species delimitation analyses for COI and 16S, and 16S bPTP. The analysis included specimens with the white morphotype of *Trapania pallida* from Ireland, white specimens with yellow appendages, rhinophores, gills, oral tentacles and tail, corresponding with the morphotype of *Trapania hispalensis*, from the Atlantic coast of France, Portugal, Spain and Morocco, as well as the Mediterranean coast of Morocco, and specimens of *T. lineata* from the Mediterranean coast of Spain and Italy (Figure 2; Table S1). The network showed 10 haplotypes, of which only two were shared between specimens, each of them by two individuals (Figure 2). One of the haplotypes were shared by a specimen of *T. lineata* with thin lines along the body, from Menorca (Spain), and a specimen with *T. hispalensis* morphotype from Capghir (Morocco). The second haplotype was shared by two specimens of *T. lineata* collected in Girona (Spain). The overall network showed a lack of geographic structure, with no apparent relationship between the *T. hispalensis* morphotype and *T. lineata*, as well as the different locations. However, the haplotypes corresponding to the specimens from Ireland were grouped in a same clade, being the morphotype of *T. pallida* the most distant with *T. hispalensis* morphotype and *T. lineata*, with six mutation sites between both groups.

3.3 | Morphological analyses

Results of morphological analyses are shown in Appendix S1, including redescription of species, drawings of internal organs (Figure S1) and scanning electron photographs of radulae, labial cuticles and penises (Figures S2–S4). Morphological analyses supported the results obtained based on molecular data. The study of the external

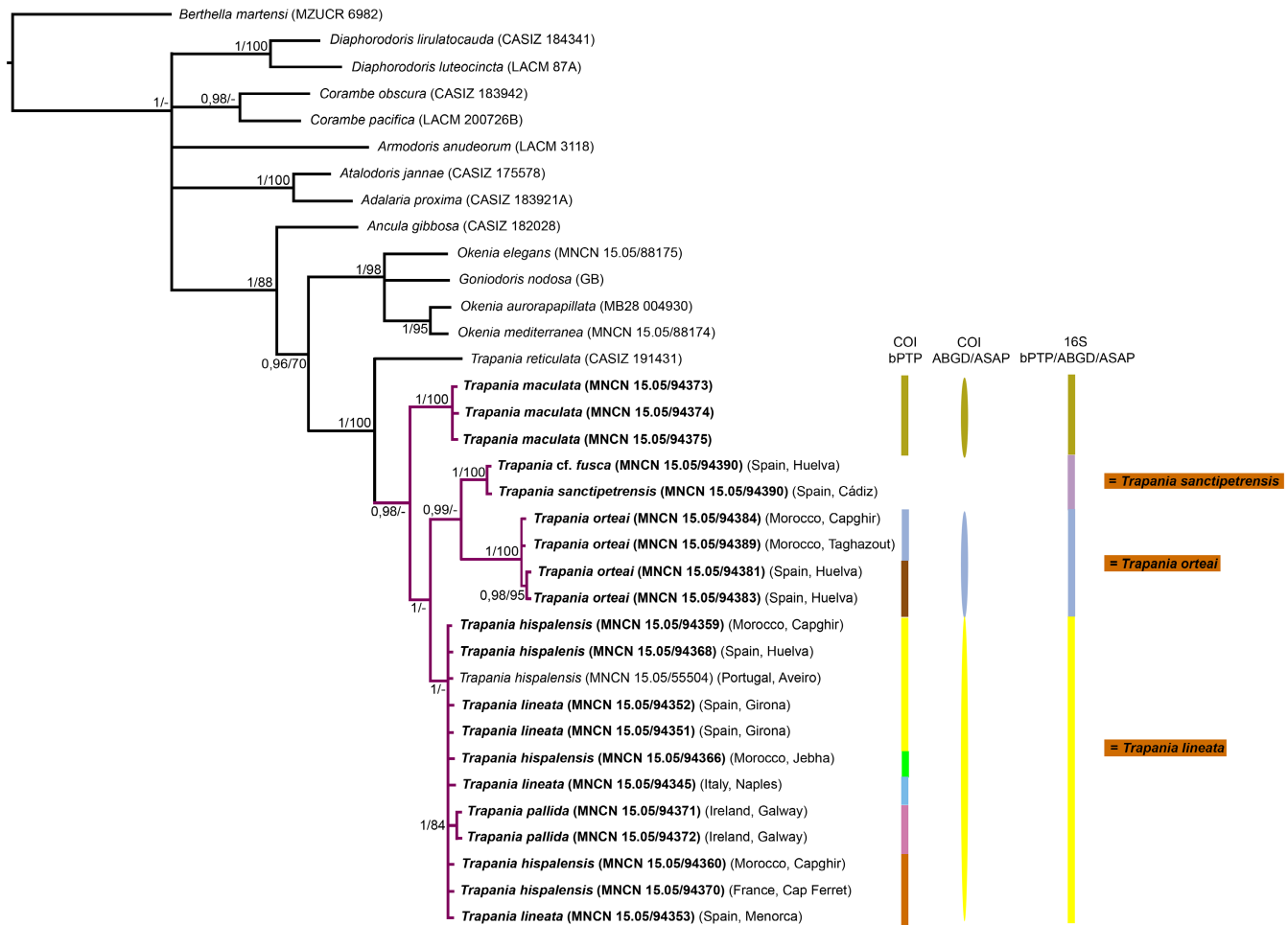


FIGURE 1 Phylogenetic relationships (BI/ML) based on the concatenated mitochondrial (COI and 16S) and nuclear (H3) genes. Purple branches represent *Trapania* taxa. *Trapania* species newly sequenced are in bold. Different colours highlighted in bPTP, ABGD and ASAP species delimitation analyses for COI and 16S represent potential different taxa

morphology and internal anatomy of *Trapania lineata*, and the synonymized *Trapania hispalensis* and *Trapania pallida* showed that the main difference between them was the colour pattern (Figure 3), which turned to be a colour variability within the senior synonym species *T. lineata*. The radula morphology showed some intraspecific variations not only amongst specimens, but also between different teeth within the same radula (see Systematic Results: Appendix S1). Another species with intraspecific variation in the radula was *Trapania maculata*. However, this species, like *Trapania ortei*, did not show remarkable variations in the colour pattern (Figure 4a–d). *Trapania ortei* showed slight differences in the size of the spots that cover the body, being larger in some specimens than in others. The second species with differences in colour pattern was *Trapania sanctipetrensis* (Figure 4e–f). We studied one specimen from Huelva that matched with the poor original description of *Trapania fusca*. However, morphological and molecular results showed that the specimen from Huelva was *T. sanctipetrensis*,

whose studied specimen was collected in the type locality, Sancti Petri, Cádiz (Spain). This work has been registered under Zoobank Accession: urn:lsid:zoobank.org:pub:A0417F75-5074-4CE2-9A47-2BEE96BA5FFF.

4 | DISCUSSION

The colour pattern is one of the most common features used to identify nudibranchs species (Korshunova et al., 2020; Layton et al., 2018; Padula et al., 2016). However, the incorporation of molecular analyses has shown that morphology can be misleading. Cryptic or pseudocryptic species have been discovered thanks to molecular analyses in several groups of nudibranchs, showing the existence of species that had gone unnoticed due to the great morphological similarity with another species (Korshunova et al., 2019; Layton et al., 2018; Martín-Hervás et al., 2021; Pola et al., 2014, 2019; Sørensen et al., 2020; Toms et al., 2021). On the contrary, intraspecific variability within the same

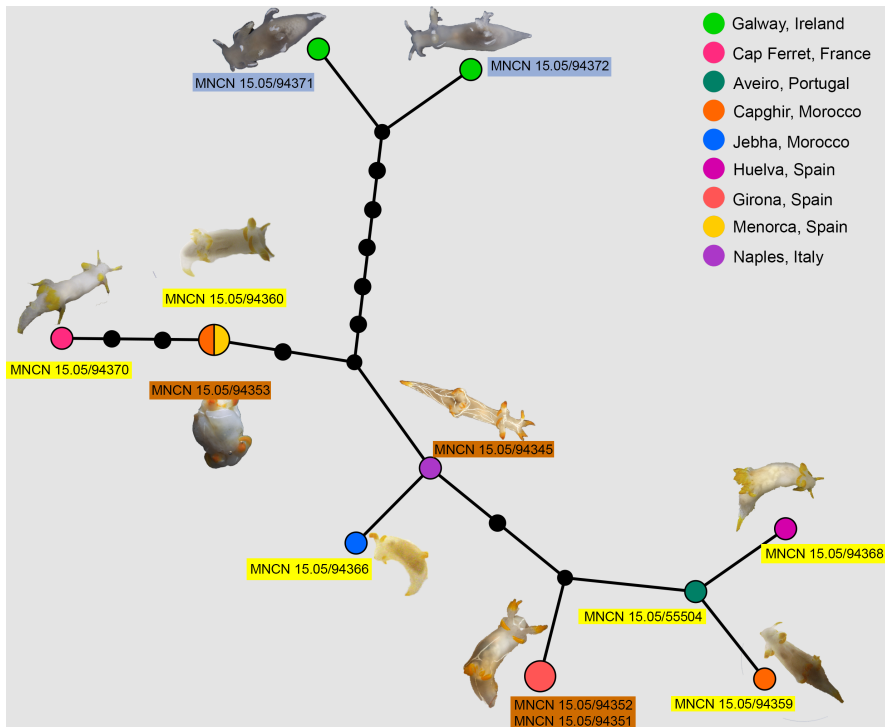


FIGURE 2 MJ haplotype network analysis based on the COI using PopArt, including sequences of 12 specimens of *Trapania lineata*. Lines between black dots represent one mutation and black dots represent hypothetical haplotypes. Each coloured circle represents a unique haplotype, and the size of each circle indicates how many specimens share that haplotype. Different colours represent geographic locations. Vouchers within orange, blue and yellow rectangle indicates specimens previously identified as *Trapania lineata*, *Trapania pallida* and *Trapania hispalensis*, respectively

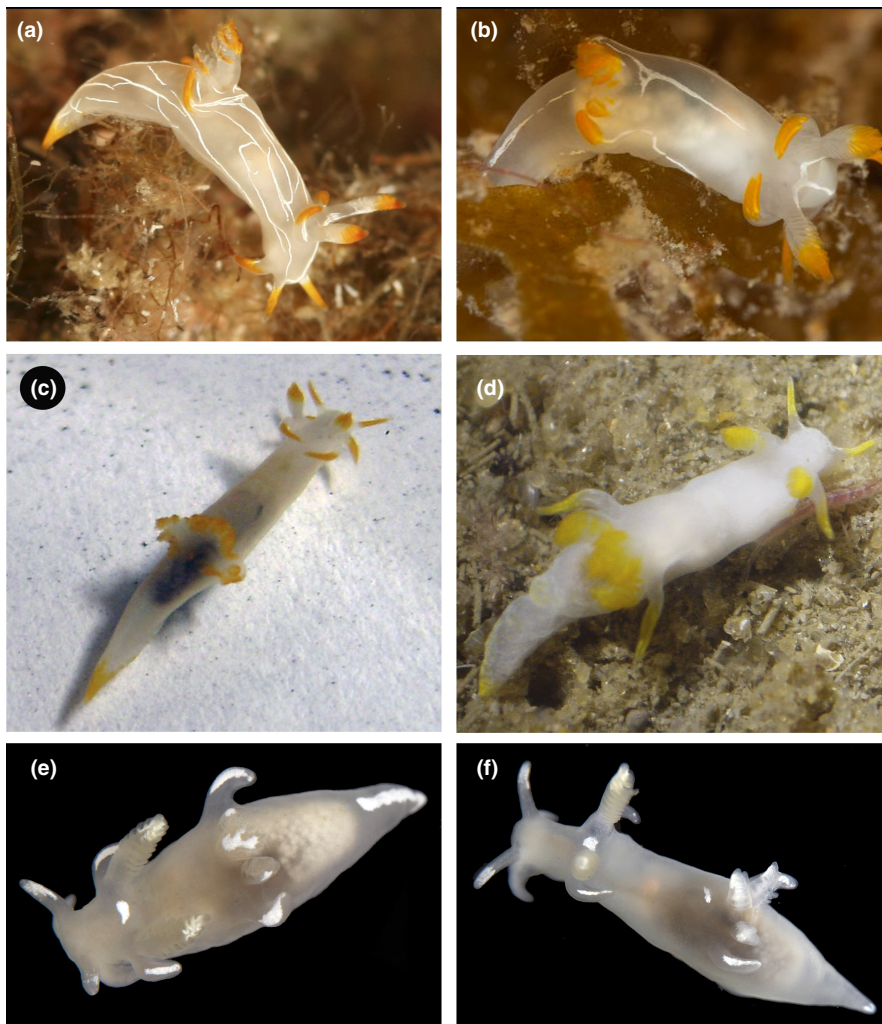


FIGURE 3 Photograph of *Trapania lineata* Haefelfinger, 1960 living animal. (a) Specimen from Naples (Italy) photographed by M. Pola (MNCN 15.05/94345). (b) Specimen from Girona (Spain) photographed by E. Madrenas (MNCN 15.05/94352). (c) Specimen from M'diq (Morocco) photographed by N. Tamsouri (MNCN 15.05/94361). (d) Specimen from Cap Ferret (France) photographed by M. Poddubestkaia (MNCN 15.05/94370). (e) Specimen from Galway (Ireland) photographed by B. Picton (MNCN 15.05/94371). (f) Specimen from Galway (Ireland) photographed by B. Picton (MNCN 15.05/94372)

species has been observed and molecularly supported, showing that the same species can present varied morphotypes (Almada et al., 2016; Araujo et al., 2022; Layton et al., 2018; Padula et al., 2016; Paz-Sedano et al., 2017; Sørensen et al., 2020). As a consequence, the reliability of using colour pattern as a diagnostic character has already been questioned within nudibranchs (Layton et al., 2018). In addition to this question, we here confirm the theories of some authors that the colour pattern of European species of *Trapania* should be taken with caution (Edmunds, 2009; Rudman, 1987), proving the intraspecific colour variability of the species *Trapania lineata* and *Trapania sanctipetrensis*.

Even though the bPTP species delimitation analysis based on COI sequences divided *T. lineata* into five different taxa and *Trapania ortei* into two taxa, ABGD and ASAP analyses on COI, and bPTP, ABGD and ASAP analyses on 16S supported the validity of both species. bPTP analysis has previously shown to over-splitting clades recognized in phylogenetic analyses (Layton et al., 2018; Paz-Sedano et al., 2021). In addition, results are also supported by COI *p*-distances. Some nudibranchs have shown interspecific genetic distances as low as 4.3% in *Polycera* (Sørensen et al., 2020) or even a value of 3.6% in *Halgerda* (Tibirić et al., 2018). The value here obtained is notably lower than these limits, with a higher COI

p-distance within *T. lineata* and within *T. ortei* of 2.1% (Table 2). Notably, the higher value within *T. lineata* is the result of comparing specimens of the synonymized *Trapania pallida*, from Ireland with the remaining southernmost specimens. In addition, Irish specimens were the most distant in the haplotype network and clustered in the phylogenetic tree. These results reflect a reduced gene flow between populations, which may show an incipient speciation of northern specimens, where a lineage division is incomplete due to insufficient time of reproductive isolation. However, since *Trapania pallida* has recently reported on the Mediterranean coasts of France (Canes (Horst, 2010) and Cote d'Azur (Meudic et al., 2016)) and Italy (La Spezia (Trainito et al., 2018)), the isolation could break down and this morphotype could not evolve as a different species. It would be interesting to perform haplotypes network analyses including specimens of *Trapania pallida* morphotype collected in the Mediterranean Sea to clarify this possible incipient speciation of the morphotype. In *T. ortei*, the maximum *p*-distance corresponds to the comparison of specimens from Morocco vs. specimens from Spain. However, in the present study, we only included sequences of specimens from two different populations. Therefore, the taxon sampling is insufficient to consider an incipient speciation.



FIGURE 4 Photographs of living animals. (a) *Trapania maculata* (MNCN 15.05/94373) photographed by M. Pontes. (b) *Trapania maculata* (MNCN 15.05/94376) photographed by J. Pereira. (c) *Trapania ortei* (MNCN 15.05/94389) photographed by N. Tamsouri. (d) *Trapania ortei* (MNCN 15.05/94381) photographed by J. F. Martín. (e) *Trapania sanctipetrensis* (MNCN 15.05/94390) photographed by J. F. Martín. (f) *Trapania sanctipetrensis* (MNCN 15.05/94391) photographed by J. A. Cuesta

Regarding the morphology of the species, the North Eastern Atlantic-Mediterranean species of *Trapania* here included are well-described in their original description, as well as by other authors that posteriorly recorded these species (Ballesteros, 1985; Brown & Picton, 1976; Cervera & García-Gómez, 1989a, 1989b; Cervera et al., 2000; Gavaia et al., 2004; Haefelfinger, 1960; Kress, 1968, 1970; Ortea et al., 1989; Tamsouri, 2014; Thompson & Brown, 1984). Our specimens match these descriptions (see Appendix S1). In addition, our study confirms the variability in the colour pattern of *T. lineata* and *T. sanctipetrensis* and adds new details of the penial spines in the species *T. oratei*, *T. sanctipetrensis* and *T. tartanella*. The presence of elongated, pointed penial spines has already been reported for these species (Cervera & García-Gómez, 1989b; Cervera et al., 2000; Ortea et al., 1989), including the difference in size between the spines located at the base and at the most distal part of the penis in *T. oratei* (Cervera & García-Gómez, 1989b). However, the change in elongated spines to rectangular spines with several cusps was not observed in any of them.

The evidence of the colour variability within *Trapania* species may rekindle some doubts about the synonymy of *T. fusca*, *T. tartanella* and *T. graeffei*, which most significant difference that supported the validity of these species was the colour pattern (Doneddu et al., 2020; Ortea et al., 1989). In addition, *T. sanctipetrensis* could be added to this possible synonymy due to the great similarity of its radula with *T. tartanella* and *T. graeffei* (Ortea et al., 1989). The four of them share a brown colour pattern with different intensity. However, in the present study, only a specimen *T. sanctipetrensis* was found suitable for molecular analyses collected from the type locality. Despite the concordance of the specimen collected from Huelva (Spain) with the species *T. fusca*, we preferred to retain the name of *T. sanctipetrensis* instead of considering it synonym of *T. fusca* until specimens of *T. fusca* are found in the type locality (Arcachon, France). Similarly, no anatomical differences were found between the species *T. sanctipetrensis* and the preserved neotype of *T. tartanella*. However, the neotype of *T. tartanella* was initially preserved in formalin; therefore, it does not allow molecular studies. Furthermore, *T. tartanella* was originally described from Naples (Italy), Mediterranean Sea, (Ihering, 1886) and the neotype was collected in Asturias (Spain) (Ortea et al., 1989). Although our study shows that the differences between the species cannot be based solely on the colour pattern, and the external morphology and internal anatomy do not show great differences, we prefer to keep the species *T. fusca*, *T. tartanella*, *T. sanctipetrensis* and *T. graeffei* as valid species until new specimens from the type locality of these species are collected and sequenced.

It is also worth mentioning that Pruvot-Fol (1954) illustrated a species of *Trapania*, *Trapania lafonti*, in her contribution to the Opisthobranch fauna from France, including a drawing of the dorsal view of the animal, teeth, penial spines and jaws. However, she did not include any description or other reference in the text, nor did she mention the authority. Pruvot-Fol (1954) cited specifically that ‘Three species have been described; having found intermediaries between two of them, I am convinced that ‘*Drepania tartanella*’ is the youngster of ‘*Drepania fusca*’. The third is also synonymous (variety of colouring). There is therefore only one species in Europe’. Immediately after, the author described *Trapania fusca*, indicating the species *Drepania graeffei* and *Drepania tartanella* as synonyms. The existence of three *Trapania* species until 1953 is also in agreement with MolluscaBase (2022), which only add *Trapania japonica* (Baba, 1935), from Japan, before that date. Therefore, the three species described up to that time by Pruvot-Fol (1954) were *T. fusca*, *T. graeffei* and *T. tartanella*, without signs of *T. lafonti*. We only found *Trapania lafonti* in this publication. It is possible that Pruvot-Fol (1954) used the name *T. lafonti* referring to *T. fusca*, alluding to the author of the species, Lafont. Therefore, *Trapania lafonti* must be considered as a not available name following the Zoological Nomenclature Code, which indicates that ‘to be available, every new name published after 1930 must be accompanied by a description or definition [...], or be accompanied by a bibliographic reference [...] or be proposed expressly as a new replacement name (nomen novum) for an available name’ (ICZN, 2000).

In this study, we here carried out a preliminary attempt to clarify the biodiversity of *Trapania* species that inhabit the templated coast of North Eastern Atlantic Ocean and the Mediterranean. We confirmed the validity of *T. lineata*, *T. maculata* and *T. oratei*, which inhabit both the Atlantic Ocean and the Mediterranean Sea, crossing the Strait of Gibraltar (Ballesteros et al., 2019; Cervera & García-Gómez, 1989b; Haefelfinger, 1960; Kress, 1968; Ortea & Urgorri, 1981). However, the species *Trapania hispalensis*, *T. lineata* and *T. pallida* belong to the same taxa, with *Trapania lineata* as senior synonym. In addition, the validity of the species *T. fusca*, *T. graeffei*, *T. sanctipetrensis* and *T. tartanella* remains pending to sequence of specimens from the type localities in future studies.

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REFERENCES

- Alfaro, M. E., Zoller, S., & Lutzoni, F. (2003). Bayes or bootstraps? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution*, *20*, 255–266. <https://doi.org/10.1093/molbev/msg028>
- Almada, F., Levy, A., & Robalo, J. I. (2016). Not so sluggish: the success of the *Felimare picta* complex (Gastropoda, Nudibranchia) crossing Atlantic biogeographic barriers. *PeerJ*, *4*, e1561. <https://doi.org/10.7717/peerj.1561>
- Araujo, A. K., Pola, M., Malaquias, M. A. E., Ballesteros, M., Vitale, F., & Cervera, J. L. (2022). Molecular phylogeny of European Runcinida (Gastropoda, Heterobranchia): the discovery of an unexpected pool of species complex, with special reference to the case of *Runcina coronata* (Quatrefages, 1844). *Zoological Journal of the Linnean Society*, *194*(3), 761–788. <https://doi.org/10.1093/zoolin/zlab041>
- Ballesteros, M. (1985). *Contribución al conocimiento de los Sacoglossos y Nudibranchios (Mollusca: Opisthobranchia). Estudio anatómico, sistemático y faunístico de las especies del Mediterráneo español*. Doctoral dissertation, Universitat de Barcelona, Centre de Publicacions. Intercanvi Científic i Extensió Universitària.
- Ballesteros, M. J., Pontes, M., & Madrenas, E. (2019). *Els nudibrànquies del mar català*. Brau edicions.
- Brown, G. H., & Picton, B. E. (1976). *Trapania maculata* Haefelfinger, a doridacean nudibranch new to the British fauna. *Journal of Conchology*, *29*(1), 63–65.
- Cattaneo-Viatti, R., & Thompson, T. E. (1989). Mediterranean opisthobranch molluscs: a zoogeographic approach. *Bolettino Malacologico, Milano*, *25*, 183–204.
- Cervera, J. L., & García-Gómez, J. C. (1989a). Redescrición de *Trapania maculata* Haefelfinger, 1960 (Gastropoda, Nudibranchia). *Bollettino Malacologico*, *24*, 189–204.
- Cervera, J., & García-Gómez, J. C. (1989b). Dos nuevas especies de *Trapania* Pruvot-Fol, 1931 (Gastropoda: Nudibranchia) del sur de España. *Bollettino Malacologico*, *24*, 189–204.
- Cervera, J. L., García-Gómez, J. C., & Megina, C. (2000). A new species of *Trapania* Pruvot-Fol, 1931 from the Bay of Cadiz. With remarks on other *Trapania* species: (Nudibranchia: Goniadorididae). *Ophelia*, *52*(1), 17–24. <https://doi.org/10.1080/00785236.1999.10409416>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- Colgan, D. J., Mclachlan, A., Wilson, G. D. F., Livingston, S., Macaranas, J., Edgecombe, G. D., Macaranas, J., Cassis, G., & Gray, M. R. (1998). Molecular phylogenetics of the Arthropoda: Relationships based on histone H and U2 snRNA DNA sequences. *Australian Journal of Zoology*, *46*, 419–437. <https://doi.org/10.1071/ZO98048>
- Doneddu, M., Trainito, E., & Furfaro, G. (2020). *Trapania graefei* (Bergh, 1881) (Gastropoda, Nudibranchia) is a valid Mediterranean species. *Bollettino Malacologico*, *56*, 86–90.
- Edmunds, M. (2009). Opisthobranchiate Mollusca from Ghana: Goniadorididae. *Journal of Conchology*, *40*(1), 37–54.
- 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. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.
- Gavaia, C., Malaquias, M. A. E., Calado, G., & Urgorri, V. (2004). New records of Portuguese opisthobranch molluscs. *Journal of Conchology*, *38*, 101–118.
- Gosliner, T. M., & Fahey, S. J. (2008). Systematics of *Trapania* (Mollusca: Nudibranchia: Goniadorididae) with descriptions of 16 new species. *Systematics and Biodiversity*, *6*(1), 53–98. <https://doi.org/10.1017/S1477200007002587>
- Haefelfinger, H. R. (1960). Neue und wenig bekannte Opisthobranchier der Gattungen *Trapania* und *Caloria* aus der Bucht von Villefranche-sur-Mer. *Revue Suisse de Zoologie*, *67*, 226–238.
- Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, *42*, 182–192. <https://doi.org/10.1093/sysbio/42.2.182>
- Horst, D. (2010). *Trapania pallida* from French Mediterranean. [Message in] *Sea Slug Forum*. Australian Museum, Sydney. <http://www.seaslugforum.net/find/23180>
- ICZN (2000). *International code of zoological nomenclature* (4th ed.). International Trust for Zoological Nomenclature.
- Ihering, H. (1886). Beiträge zur kenntnis der Nudibranchien des Mittelmeeres. Part 2. *Malakozoologische Blätter N.F.*, *8*, 12–48.
- Just, H., & Edmunds, M. (1985). North Atlantic nudibranchs (Mollusca) seen by Henning Lemche with additional species from the Mediterranean and the North East Pacific. *Ophelia Supplementum*, *2*, 1–170.
- Korshunova, T., Malmberg, K., Prkić, J., Petani, A., Fletcher, K., Lundin, K., & Martynov, A. (2020). Fine-scale species delimitation: Speciation in process and periodic patterns in nudibranch

- diversity. *ZooKeys*, 917, 15–50. <https://doi.org/10.3897/zookeys.917.47444>
- Korshunova, T., Picton, B., Furfaro, G., Mariottini, P., Pontes, M., Prkić, J., Fletcher, K., Malmberg, K., Lundin, K., & Martynov, A. (2019). Multilevel fine-scale diversity challenges the 'cryptic species' concept. *Scientific Reports*, 9, 1–23. <https://doi.org/10.1038/s41598-019-42297-5>
- Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*, 35, 44534455. <https://doi.org/10.1093/bioinformatics/btz305>
- Kress, A. (1968). *Trapania pallida* sp. nov. (Opisthobranchia, Gastropoda), a genus new to Britain. *Journal of Molluscan Studies*, 38(2), 161–165. <https://doi.org/10.1093/oxfordjournals.mollus.a065034>
- Kress, A. (1970). A new record of *Trapania pallida* (Opisthobranchia, Gastropoda) with a description of its reproductive system and a comparison with *T. fusca*. *Journal of Molluscan Studies*, 39(2–3), 111–116. <https://doi.org/10.1093/oxfordjournals.mollus.a065085>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lafont, A. (1874). Description d'un nouveau genre de nudibranche des côtes de France. *Journal de Conchyliologie*, 22(4), 369–370.
- Layton, K. K., Gosliner, T. M., & Wilson, N. G. (2018). Flexible colour patterns obscure identification and mimicry in Indo-Pacific *Chromodoris* Nudibranchs (Gastropoda: Chromodorididae). *Molecular Phylogenetics and Evolution*, 124, 27–36. <https://doi.org/10.1016/j.ympev.2018.02.008>
- Leigh, J. W., & Bryant, D. (2015). Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Marcus, E. R. (1957). On opisthobranchia from Brazil (2). *Journal of Linnean Society of London*, 43(292), 390–486. <https://doi.org/10.1111/j.1096-3642.1957.tb01559.x>
- Martín-Hervás, M. D. R., Carmona, L., Malaquias, M. A. E., Krug, P. J., Gosliner, T. M., & Cervera, J. L. (2021). A molecular phylogeny of *Thuridilla* Bergh, 1872 sea slugs (Gastropoda, Sacoglossa) reveals a case of flamboyant and cryptic radiation in the marine realm. *Cladistics*, 37(6), 647–676.
- Meudic, J., Sittler, A. P., & Horst, D. (2016). *Trapania pallida* Kress, 1968. DORIS. <http://dorisffesmfr/ref/specie/1484>
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 November 2010, New Orleans, LA, USA. INSPEC Accession Number 11705685. IEEE. <https://doi.org/10.1109/GCE.2010.5676129>
- MolluscaBase eds. (2022). *MolluscaBase. Trapania Pruvot-Fol, 1931*. Accessed through: World Register of Marine Species at: <https://marinespecies.org/aphia.php?p=taxdetails&id=138043>
- Moro, L., & Ortea, J. (2015). Nuevos taxones de babosas marinas de las islas Canarias y de Cabo Verde (Mollusca: Heterobranchia). *Vieraea*, 43, 21–86.
- Nordsieck, F. (1972). *Die europäischen Meeresschnecken (Opisthobranchia mit Pyramidellidae; Rissoacea), Vom Eismeer bis Kapverden, Mittelmeer und Schwarzes Meer*. Gustav Fischer Verlag.
- Ortea, J., Quero, A., Rodríguez, G., & Valdés, A. (1989). Redescrpción de *Trapania tartanella* (Ihering, 1986) (Mollusca: Nudibranchia). *Bollettino Malacologico*, 25(5–8), 241–246.
- Ortea, J., & Urgorri, V. (1981). Opisthobranchios nuevos para el litoral ibérico colectados en Galicia. I. *Boletín del Instituto Español de Oceanografía*, 6, 49–60.
- Padula, V., Bahia, J., Stöger, I., Camacho-García, Y., Malaquias, M. A. E., Cervera, J. L., & Schrödl, M. (2016). A test of color-based taxonomy in nudibranchs: Molecular phylogeny and species delimitation of the *Felimida clenchi* (Mollusca: Chromodorididae) species complex. *Molecular Phylogenetics and Evolution*, 103, 215–229. <https://doi.org/10.1016/j.ympev.2016.07.019>
- Palumbi, S. R. (1996). Nucleic acids II: The polymerase chain reaction. *Molecular Systematics*, 205–247.
- Paz-Sedano, S., Díaz-Agras, G., Gosliner, T. M., & Pola, M. (2021). Revealing morphological characteristics of Goniodorididae genera (Mollusca: Nudibranchia). *Organisms Diversity & Evolution*, 22(1), 93–116. <https://doi.org/10.1007/s13127-021-00508-w>
- Paz-Sedano, S., Ortigosa, D., & Pola, M. (2017). A new *Okenia* Menke, 1830 from the Azores Island, Portugal. *Spixiana*, 40(1), 13–22.
- Paz-Sedano, S., Wilson, N. G., Carmona, L., Gosliner, T. M., & Pola, M. (2021). An ocean yet to be discovered: increasing systematic knowledge of Indo-Pacific *Okenia* Menke, 1830 (Nudibranchia: Goniodorididae). *Invertebrate Systematics*, 35(7), 797–825. <https://doi.org/10.1071/IS20088>
- Pola, M., Paz-Sedano, S., Macali, A., Minchin, D., Marchini, A., Vitale, F., Licchelli, C., & Crocetta, F. (2019). What is really out there? Review of the genus *Okenia* Menke, 1830 (Nudibranchia: Goniodorididae) in the Mediterranean Sea with description of two new species. *PLoS One*, 14(5), e0215037. <https://doi.org/10.1371/journal.pone.0215037>
- Pola, M., Roldan, P., & Padilla, S. (2014). Molecular data on the genus *Okenia* (Nudibranchia: Goniodorididae) reveal a new cryptic species from New South Wales (Australia). *Journal of the Marine Biological Association of the United Kingdom*, 94(3), 587–598. <https://doi.org/10.1017/S0025315413001793>
- Pruvot-Fol, A. (1931). Notes de systématique sur les opisthobranches. *Bulletin du Muséum National D'histoire Naturelle, Paris*, 3(3), 308–316.
- Pruvot-Fol, A. (1954). *Faune de France. Mollusques Opisthobranches*. Lechevalier.
- Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: Assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2), 609–620. <https://doi.org/10.1111/1755-0998.13281>
- Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21(8), 1864–1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x>
- Rambaut, A. (2009). *FigTree v1.3.1: Tree Figure Drawing Tool*. <http://tree.bio.ed.ac.uk/software/figtree/>
- Rudman, W. B. (1987). The genus *Trapania* (Nudibranchia: Goniodorididae) in the Indo-West Pacific. *Journal of Molluscan Studies*, 53(2), 189–212. <https://doi.org/10.1093/mollus/53.2.189>
- Sabelli, B., Giannuzzi-Savelli, R., & Bedulli, D. (1990). *Catalogo annotato dei molluschi marini del Mediterraneo. Annotated checklist of Mediterranean marine mollusks, I*. Società Italiana di Malacologia. Edizioni Libreria Nauralistica Bolognese.
- Sammut, C., & Perrone, A. (1998). A preliminary check-list of Opisthobranchia (Mollusca, Gastropoda) from the Maltese Islands. *Basteria*, 62(5–6), 221–240.

- Schmekel, L., & Portmann, A. (1982). *Opisthobranchia des Mittelmeeres*. Springer-Verlag.
- Schwarz, G. (1978). Estimating the dimension of a model. *Annals of Statistics*, 6(2), 461–464. <https://doi.org/10.1214/aos/1176344136>
- Sørensen, C. G., Rauch, C., Pola, M., & Malaquias, M. A. E. (2020). Integrative taxonomy reveals a cryptic species of the nudibranch genus *Polycera* (Polyceridae) in European waters. *Journal of the Marine Biological Association of the United Kingdom*, 100(5), 733–752. <https://doi.org/10.1017/S0025315420000612>
- Tamsouri, M. N. (2014). *La biodiversité d'un groupe de mollusques marins peu exploré au Maroc: Les Limaces de mer (Gastropoda, Heterobranchia)*. PhD thesis, Université Ibn Zohr, Faculté des Sciences, Agadir, Morocco. Unpublished.
- Templado, J., Luque, A. A., & Moreno, D. (1988). Nuevas aportaciones al conocimiento de los opisthobranchios del sureste español. *Iberus*, 8, 15–23.
- Thompson, T. E., & Brown, G. H. (1984). *Biology of opisthobranch molluscs* (vol. II). The Ray Society.
- Tibiricá, Y., Pola, M., & Cervera, J. L. (2018). Systematics of the genus *Halgerda* Bergh, 1880 (Heterobranchia: Nudibranchia) of Mozambique with descriptions of six new species. *Invertebrate Systematics*, 32(6), 1388–1421. <https://doi.org/10.1071/IS17095>
- Toms, J. A., Pola, M., von der Heyden, S., & Gosliner, T. M. (2021). Disentangling species of the genus *Limacia* OF Müller, 1781, from southern Africa and Europe using integrative taxonomical methods, with the description of four new species. *Marine Biodiversity*, 51(1), 1–31. <https://doi.org/10.1007/s12526-020-01125-0>
- Trainito, E., & Doneddu, M. (2014). *Nudibranchi del Mediterraneo* (2^a ed.). Il Castello.
- Trainito, E., Fantin, M., & Furfaro, G. (2018). *Trapania pallida* Kress, 1968 (Gastropoda, Nudibranchia): First record for Italian waters and new additional notes on its diet and on Mediterranean records. *Studia Marina*, 31(2), 32–37.
- Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>

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