Zootaxa 5133 (3): 383–406
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5133.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:547DC9FA-B078-49D2-85E3-8D579783AB5B

Attack on crypsis: Molecular and morphological study of *Dendrodoris* Ehrenberg, 1831 (Mollusca: Gastropoda: Nudibranchia) from the Mediterranean Sea and Northern Atlantic Ocean reinstates *Dendrodoris temarana* Pruvot-Fol, 1953

CARLES GALIÀ-CAMPS[1,2*], JUAN LUCAS CERVERA[3,4], ÁNGEL VALDÉS[5] & MANUEL BALLESTEROS[1,6]

[1]*Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Avda. Diagonal, 643, 08028 Barcelona, Spain*

[2]*Departament de Genètica, Microbiologia i Estadística, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Avinguda Diagonal 643 CP 08028, Barcelona, Spain*

[3]*Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus de Excelencia Internacional del Mar (CEIMAR), Universidad de Cádiz, Av. República Saharaui, s/n, P.O. Box 40, 11510 Puerto Real (Cádiz), Spain.*
lucas.cervera@uca.es; https://orcid.org/0000-0002-8337-2867

[4]*Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEIMAR), Universidad de Cádiz, Av. República Saharaui, s/n, P.O. Box 40, 11510 Puerto Real (Cádiz), Spain.*

[5]*Department of Biological Sciences, California State Polytechnic University Pomona, 3801 West Temple Avenue, Pomona, California 91768, USA aavaldes@cpp.edu; https://orcid.org/0000-0002-2347-4896*

[6] *mballesteros@ub.edu; https://orcid.org/0000-0001-9055-1241*

[*]*Correspondence author. cgaliacamps@gmail.com; https://orcid.org/0000-0002-0945-0249*

Abstract

The genus *Dendrodoris* is ascribed to the family Dendrodorididae, whose members are characterized lacking spicules in the dorsum as well as a radula, one of the most important taxonomic traits to distinguish and define nudibranch species. The absence of this taxonomic character makes difficult to delineate species based on morphologic traits, and consequently, several cryptic species may remain unreported resulting in a potential underestimation of the real diversity of the genus. Species delimitation analyses based on molecular data constitute an important tool to unveil cryptic species, especially when morphology may be misleading. However, for the genus *Dendrodoris*, only two molecular studies have been performed to date, both based on Indo-pacific species and the molecular marker COI. In the present study, we performed a multilocus approach using the molecular markers COI, 16S and H3 based on 47 *Dendrodoris* specimens from the North Atlantic Ocean and Mediterranean Sea. As a result, we detected monophyly for five of the previously accepted species plus the presence of an unidentified one. Using morphological and anatomical data from 14 additional individuals and a review of the literature, we verified this unidentified species is the previously synonymized species *Dendrodoris temarana* Pruvot-Fol, 1953, and we redescribe it morphologically (chromatic pattern, gills, rhinophores and anal papilla), anatomically (neural, circulatory, digestive and reproductive systems), and biologically herein. *D. temarana* is differentiated by its chromatic pattern, previously thought to be intraspecific variation of *D. grandiflora* (Rapp, 1827), *D. limbata* (Cuvier, 1804) and *D. herytra* Valdés & Ortea in Valdés, Ortea, Avila & Ballesteros, 1996, and therefore it is considered a pseudocryptic species. This study increases the number of valid species in the North Atlantic and Mediterranean to nine and confirms the importance of integrative taxonomic approaches to resolve the identification of cryptic species in complex groups.

Key words: Species complex, radula-less dorid, Dendrodorididae, chromatic pattern, biodiversity, Heterobranchia

Introduction

The existence of cryptic and pseudocryptic species is one of the most challenging phenomena that taxonomists must deal with (Bickford *et al*. 2007; Pfenninger & Schwenk 2007; Appeltans *et al*. 2012). Cryptic and pseudocryptic species are characterized by being genetically distinct but having few to no distinguishing morphological traits (Marsh *et al*. 1981). Consequently, they are difficult to detect, leading to biodiversity underestimation. As it has been demonstrated for several taxa, molecular data can be used to successfully unveil cryptic species complexes, which are particularly common in heterobranch sea slugs (Hoover *et al*. 2015; Araujo *et al*. 2019, 2021; Golestani *et al.* 2019; Pola *et al.* 2019; Galià-Camps *et al.* 2020; Martín-Hervás *et al*. 2020).

Accepted by V. Padula: 28 Mar. 2022; published: 5 May 2022

The nudibranch genus *Dendrodoris* Ehrenberg, 1831 is currently included in the superfamily Phyllidioidea Rafinesque, 1814, whose members are informally called radula-less dorids (Valdés & Gosliner 1999). The absence of radula, one of the most important taxonomic structures used in nudibranch taxonomy, makes the identification of *Dendrodoris* species difficult, which in turn resulted in several misidentifications or unconfirmed distributional data (Valdés *et al*. 1996; Brodie & Calado 2006; Zenetos *et al.* 2017). Moreover, members of this genus display high intraspecific chromatic variability and the transition from juvenile to adult may result in a change of background color (Rose 1985; Valdés *et al*. 1996). Even though there are so many external intraspecific variable features, most studies conducted to date are primarily based on morphological data (Valdés *et al*. 1996; Valdés & Gosliner 1999), with the exception of Hirose *et al*. (2015) and Park *et al*. (2019), which used molecular data to identify Japanese and Korean species. The lack of molecular studies in *Dendrodoris* is concerning, since morphological studies performed on this genus resulted in mostly conservative taxonomies, and therefore the real biodiversity of the genus may remain underestimated. Moreover, dendrodoridids display a significantly higher frequency of intracapsular development compared to other nudibranch families (Goddard 2005), and this can lead to high levels of endemism resulting from low dispersal rates due to the absence of a swimming larval phase. According to WoRMS (MolluscaBase 2022), there are eight species of *Dendrodoris* in the North Atlantic Ocean and Mediterranean Sea: *Dendrodoris limbata* (Cuvier, 1804), *D. grandiflora* (Rapp, 1827), *D. krebsii* (Mörch, 1863), *D. senegalensis* Bouchet, 1975, *D. warta* Marcus & Gallagher, 1976, *D. herytra* Valdés & Ortea in Valdés, Ortea, Ávila & Ballesteros, 1996, *D. guineana* Valdés & Ortea, 1996 and *D. magagnai* Ortea & Espinosa, 2001. In addition, *Dendrodoris fumata* (Rüppell & Leuckart, 1830) has been recorded three times in the Mediterranean, yet the reliability of these records remains controversial (Zenetos *et al.* 2017).

The objective of this study is to explore the potential presence of cryptic species of *Dendrodoris* in the Mediterranean and North Atlantic by performing the first molecular phylogenetic study for this genus in the region. This study uses the mitochondrial markers cytochrome oxidase I (COI) and ribosomal subunit 16S (16S), as well as the nuclear marker histone 3 (H3). Also, the study includes a morphological analysis of the external and internal features of specimens from the different clades recovered from the molecular phylogenetic tree, to clarify the taxonomic status of the North-Atlantic and Mediterranean species of *Dendrodoris*.

Material and methods

Material examined. A total of 61 individuals of *Dendrodoris* were sampled under stones ranging from 0 to 2 m depth in Cuba, Cape Verde, the Canary Islands, Morocco, Portugal, Andalusia (SW Spain), Galicia (NW Spain), Catalonia (NE Spain) and Italy, and preserved in EtOH 96% (Table 1). Due to sampling limitations, *D. guineana*, *D. magagnai* and *D. warta* were not included in the current study. Examined material has been deposited in the Centre de Recursos de Biologia Animal (CRBA) of the Universitat de Barcelona, Spain, the Museu de Ciències Naturals de Barcelona (MZB), Barcelona, Spain, the Senckenberg Museum of Frankfurt (SMF), Frankfurt am Main, Germany and the Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain. For molecular studies, 47 specimens were used (Table 1). Three additional sequences of the species *Doriopsilla spaldingi* Valdés & Behrens, 1998 were retrieved from GenBank to root the tree as an outgroup taxon.

Extraction, amplification and DNA sequencing. Tissue was obtained from the foot of the preserved animals. The E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc) was used for DNA extraction. Partial sequences of the genes were amplified by PCR using the primers L1490 and H2198 for COI (Folmer *et al.* 1994) 16S ar-L and 16S br-H for 16S (Palumbi *et al*. 2002) and H3AD5'3' and H3BD5'3' for H3 (Colgan *et al*. 1998). In cases when the Folmer's primers failed to amplify COI, the primer NANCY (Simon *et al*. 1994) was used instead of H2198. PCRs were performed in reactions of 10 µL, containing 5 µL of REDExtract-N-Amp™ PCR ReadyMix™ (Merck KGaA, Darmstadt, Germany), 0.5 µL 10 µM of forward and reverse primers, 1 µL of genomic DNA, and 3 µL miliQ water. Amplification of the three genes was performed by pre-heating 5 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 42º–45°C, and 45 seconds at 72°C, with a final elongation phase of 5 minutes at 72°C. The S1000 thermal cycler hardware (Bio-Rad Laboratories, Inc., Hercules, USA) was used to carry out the PCRs. Amplification performance was corroborated by electrophoresis gel and successfully amplified were purified by mixing 2 µL of ExoSAP-IT (Thermo Fisher Scientific Inc, Waltham, USA) with the total PCR product. Sequencing was carried out at the UB Scientific-Technical Services (CCiTUB) or Macrogen Inc. (Madrid, Spain).

Phylogenetic analyses. DNA sequences were assembled with SeqMan (Swindell & Plasterer 1997) and edited

TABLE 1. Specimens used in current study. Collection site, region (EATL = East Atlantic, WATL = West Atlantic, MED = Mediterranean), collection date, voucher codes and GenBank accession numbers. Data of the neotype and new sequences obtained in this study indicated in bold.

Species name	Collection site	Region	Collection Date	Voucher code	COI	16S	H3
Dendrodoris grandiflora	Cádiz, Spain	EATL	07/04/2017	MZB2020-1168	**MW194016**	**MW194922**	**MW200262**
Dendrodoris grandiflora	Cádiz, Spain	EATL	02/11/2017	MZB2020-1169	**MW194018**	**MW194921**	**MW200264**
Dendrodoris grandiflora	Begur, Spain	MED	04/04/2016	MZB2020-1166	**MW194012**	**MW194925**	**MW200256**
Dendrodoris grandiflora	Cadaqués, Spain	MED	24/05/2016	MZB2020-1167	**MW194013**	-	**MW200257**
Dendrodoris grandiflora	Palamós, Spain	MED	28/05/2015	MZB2020-1165	**MW194011**	**MW194926**	**MW200255**
Dendrodoris herytra	O Segaño, Spain	EATL	23/05/2017	CRBA-92750	**MW194025**	**MW194915**	**MW200270**
Dendrodoris krebsii	Bahía cochinos, Cuba	WATL	16/07/2008	MNCN-15.05/92210	**MW194026**	**MW194914**	**MW200271**
Dendrodoris krebsii	Bahía cochinos, Cuba	WATL	18/07/2008	MNCN-15.05/92211	**MW194027**	**MW194913**	**MW200272**
Dendrodoris krebsii	Bahía cochinos, Cuba	WATL	18/07/2008	MNCN-15.05/92212	**MW194028**	**MW194912**	**MW200273**
Dendrodoris krebsii	Bahía cochinos, Cuba	WATL	18/07/2008	MNCN-15.05/92213	-	**MW194911**	**MW200274**
Dendrodoris limbata	Trafalgar, Spain	EATL-MED	22/01/2008	MNCN-15.05/92214	-	**MW194898**	-
Dendrodoris limbata	Begur, Spain, Spain	MED	15/11/2015	MZB2020-1172	**MW194030**	**MW194909**	-
Dendrodoris limbata	Cadaqués, Spain	MED	24/05/2015	MZB2020-1176	-	**MW194901**	-
Dendrodoris limbata	Palamós, Spain	MED	25/05/2016	MZB2020-1177	-	**MW194897**	-
Dendrodoris limbata	Port de la Selva, Spain	MED	31/10/2015	MZB2020-1173	**MW194031**	**MW194908**	**MW200276**
Dendrodoris limbata	Port Lligat, Spain	MED	31/03/2015	MZB2020-1171	**MW194029**	**MW194910**	**MW200275**
Dendrodoris limbata	Taranto, Italy	MED	09/04/2016	MZB2020-1175	**MW194009**	-	**MW200253**
Dendrodoris limbata	Taranto, Italy	MED	09/04/2016	MZB2020-1174	**MW194032**	-	**MW200277**
Dendrodoris senegalensis	Murdeira, Cabo Verde	EATL	01/09/2003	MNCN-15.05/92217	**MW194038**	**MW194907**	**MW200286**
Dendrodoris senegalensis	Rabo de Junco, Cabo Verde	EATL	06/04/2014	MNCN-15.05/92215	**MW194033**	**MW194906**	**MW200279**
Dendrodoris senegalensis	Rabo de Junco, Cabo Verde	EATL	06/04/2014	MNCN-15.05/92216	**MW194039**	-	**MW200278**
Dendrodoris senegalensis	Serra Negra, Cabo Verde	EATL	05/04/2014	MNCN-15.05/92218	-	**MW194905**	**MW200280**
Dendrodoris temarana	Cádiz, Spain	EATL	07/04/2016	MZB2020-1170	-	-	**MW200260**
Dendrodoris temarana	Cádiz, Spain	EATL	07/04/2017	CRBA-92736	**MW194019**	-	**MW200284**
Dendrodoris temarana	Cádiz, Spain	EATL	07/04/2017	CRBA-92735	**MW194036**	**MW194900**	**MW200285**
Dendrodoris temarana	Cádiz, Spain	EATL	02/11/2017	CRBA-92747	**MW194037**	**MW194899**	**MW200265**
Dendrodoris temarana	Calhau, Cabo Verde	EATL	10/07/2013	MNCN-15.05/200125	**MW194035**	**MW194902**	**MW200283**
Dendrodoris temarana	Canary Islands, Spain	EATL	25/01/2017	CRBA-92746	**MW194010**	**MW194927**	**MW200254**

......continued on the next page

TABLE 1. (Continued)

Species name	Collection site	Region	**Collection Date**	**Voucher code**	**COI**	**16S**	**H3**
Dendrodoris temarana	Canary Islands, Spain	EATL	25/01/2017	CRBA-92745	**MW194024**	**MW194916**	**MW200269**
Dendrodoris temarana	Cape Ghir, Morocco	EATL	22/06/2009	MNCN- 15.05/94682	**MZ710317**	**MZ429959**	**MZ713161**
Dendrodoris temarana	Cape Ghir, Morocco	EATL	08/10/2009	MNCN- 15.05/94683	**MZ710315**	**MZ429957**	**MZ713159**
Dendrodoris temarana	Cape Ghir, Morocco	EATL	27/04/2010	MNCN- 15.05/94684	**MZ710316**	**MZ429958**	**MZ713160**
Dendrodoris temarana	Estansinha, Cabo Verde	EATL	01/04/2009	MNCN-15.05/92209	**-**	**MW194904**	**MW200281**
Dendrodoris temarana	Porto Cais, Cabo Verde	EATL	05/04/2007	MNCN-15.05/92208	**MW194040**	**-**	**MW200287**
Dendrodoris temarana	Serra Negra, Cabo Verde	EATL	05/04/2014	MNCN-15.05/92207	**MW194034**	**MW194903**	**MW200282**
Dendrodoris temarana	Setúbal, Portugal	EATL	20/04/2017	CRBA-92749	**MW194017**	**-**	**MW200263**
Dendrodoris temarana	Setúbal, Portugal	EATL	20/04/2017	CRBA-92748	**-**	**MW194923**	**MW200261**
Dendrodoris temarana	**Temara, Morocco**	**EATL**	**30/03/2010**	**MNCN-15.05/200177**	**MZ710318**	**MZ429960**	**MZ713162**
Dendrodoris temarana	Cubelles, Spain	MED	30/06/2015	CRBA-92721	**MW194020**	**MW194920**	**MW200258**
Dendrodoris temarana	Cubelles, Spain	MED	30/06/2015	CRBA-92744	**MW194023**	**MW194917**	**MW200290**
Dendrodoris temarana	Cubelles, Spain	MED	14/12/2015	CRBA-92738	**MW194014**	**MW194924**	**MW200266**
Dendrodoris temarana	Cubelles, Spain	MED	15/01/2016	CRBA-92737	**MW194015**	**-**	**MW200259**
Dendrodoris temarana	Cubelles, Spain	MED	15/01/2016	CRBA-92742	**MW194041**	**MW194896**	**MW200288**
Dendrodoris temarana	Cubelles, Spain	MED	15/01/2016	CRBA-92743	**MW194021**	**MW194919**	**MW200289**
Dendrodoris temarana	Cubelles, Spain	MED	15/02/2016	CRBA-92739	**MW194043**	**MW194894**	**MW200267**
Dendrodoris temarana	Cubelles, Spain	MED	14/03/2016	CRBA-92740	**MW194022**	**MW194918**	**MW200268**
Dendrodoris temarana	Cubelles, Spain	MED	14/03/2016	CRBA-92741	**MW194042**	**MW194895**	**-**
Doriopsilla spaldingii	California, USA	PAC	-	-	KR002479	KR002427	KR002523

with MEGA7 (Kumar *et al*. 2016). All sequences were confirmed free of contamination by BLAST and submitted to GenBank under the codes provided in Table 1. MUSCLE (Edgar, 2004), implemented in MEGA7, was used to align the sequences. Evolutionary models for the three molecular markers were determined by performing the Akaike Information Criterion (AIC) implemented in JModelTest 2 (Darriba *et al*. 2015). Phylogenetic analyses were carried out for all three molecular markers independently, the mitochondrial genes concatenated, and a concatenated dataset of all three markers including all samples with at least two of the genes. Maximum-Likelihood analyses were performed with RAxML 8.0 (Stamatakis, 2014) with 10,000 replicates, random initial trees, and estimated parameters evolutionary models. Branches with bootstrap values above 70% were considered supported (Hillis & Bull 1993). Bayesian inference analyses were conducted with MrBayes 3.2.6b (Ronquist & Huelsenbeck 2003) with 10 million generations, four independent runs with sampling every 100 generations and an initial burnin of 25%. Posterior probability node values (PP) higher than 0.9 were considered supported (Huelsenbeck & Rannala 2004). The resulting trees were visualized with FigTree 1.4.2 (Rambaut 2014) and iToL (https://itol.embl.de/itol.cgi).

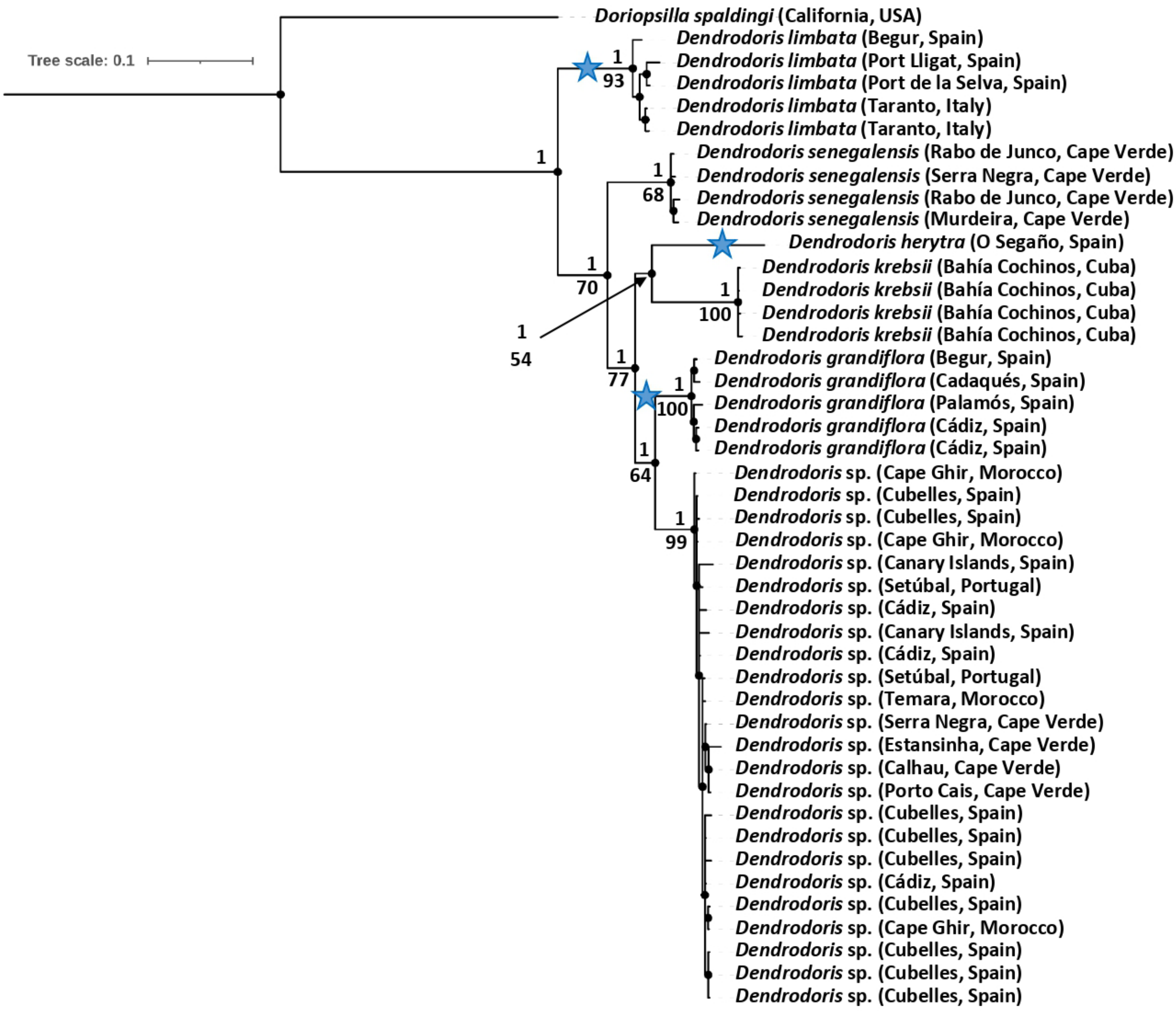


FIGURE 1. Bayesian phylogenetic tree obtained from the analysis of the COI+16S+H3 dataset. Posterior probabilities are indicated above the nodes and maximum likelihood bootstrap values below the nodes. Specimens of *Dendrodoris* sp. were previously misidentified as specimens of the species in the clades with a blue star.

Species delimitation analyses. To delineate species, we used an integrative approach including pairwise uncorrected p-distances, ABGD (Puillandre *et al*. 2012), phylogeny trees and bPTP (Zhang *et al*. 2013), as well as external and internal morphological data. Uncorrected p-distances were calculated for the COI gene with MEGA7 using a bootstrap method with 10000 replications and the uniform nucleotide substitution-rate model. The ABGD analysis was performed via web version for the COI dataset. P_{min} was established as 0.001 and P_{max} was set as 0.1, with a total of 15 steps. Simple Distance (SD), Jukes & Cantor (JC) and Kimura-80 (K80) parameters were used as nucleotide substitution models to compare each substitution model result and contrast the barcoding detection among them. The rooted COI consensus trees obtained by MrBayes were used for a bPTP analysis. The runs were executed with a total of 100,000 generations, with an initial burnin of 0.1% and a thinning value established by default as 100.

Morphological and anatomical analyses. Living specimens were photographed in their natural environment when possible and/or in the laboratory. Digital cameras (Olympus SP-350, Olympus XZ1, Olympus TG5, and Konica Minolta Dynax 7d) were used for this purpose. For the study of the internal anatomy, mid-dorsal incisions were made on preserved specimens (see taxonomic results) in order to expose the digestive and reproductive systems. Drawings of internal organs were made in their original position at freehand with the help of an Olympus model SZ-ST binocular microscope.

Results

Phylogenetic analyses. After the primer deletion, sequences were aligned obtaining fragments of 683 bp (COI), 412 bp (16S) and 345 bp (H3). The concatenation of COI+16S resulted in sequences of 1095 bp, and the COI+16S+H3 had a total of 1440 bp. The GTR+G model was given by JModelTest and chosen to conduct all the phylogenetic analyses. The topology of the COI tree resolves the species level relationships, displaying a total of six *Dendrodoris* clades, one of them corresponding to an unidentified species *Dendrodoris* sp. (Suppl. Fig.1). The 16S tree has a polytomy with four of the *Dendrodoris* species previously described and a separated clade which includes *D. grandiflora* and *Dendrodoris* sp. (Suppl. Fig. 2). The H3 topology presents a polytomy including all the specimens belonging to the genus *Dendrodoris* (Suppl. Fig. 3). The resulting analysis for the mitochondrial concatenated dataset, displayed six clades within the genus *Dendrodoris* (Suppl. Fig. 4). Lastly, the COI+16S+H3 dataset produced the same topology as the mitochondrial dataset, but with better supported nodes (Fig. 1). *D. limbata* is sister to the rest of the species with a node value of PP = 1, BS = <50. Within this second clade, *D. senegalensis* is sister to a clade containing two main groups, one comprising *D. herytra* and *D. krebsii* and another including *D. grandiflora* and *Dendrodoris* sp. (PP = 1, BS = 74). *D. herytra* and *D. krebsii* split from *D. grandiflora* and *Dendrodoris* sp. with a node value of PP = 1 and BS = 77. Finally, *D. herytra* is the sister species of *D. krebsii* (PP = 1, BS = 54), and *D. grandiflora* is the sister species of *Dendrodoris* sp. (PP=1, BS = 64).

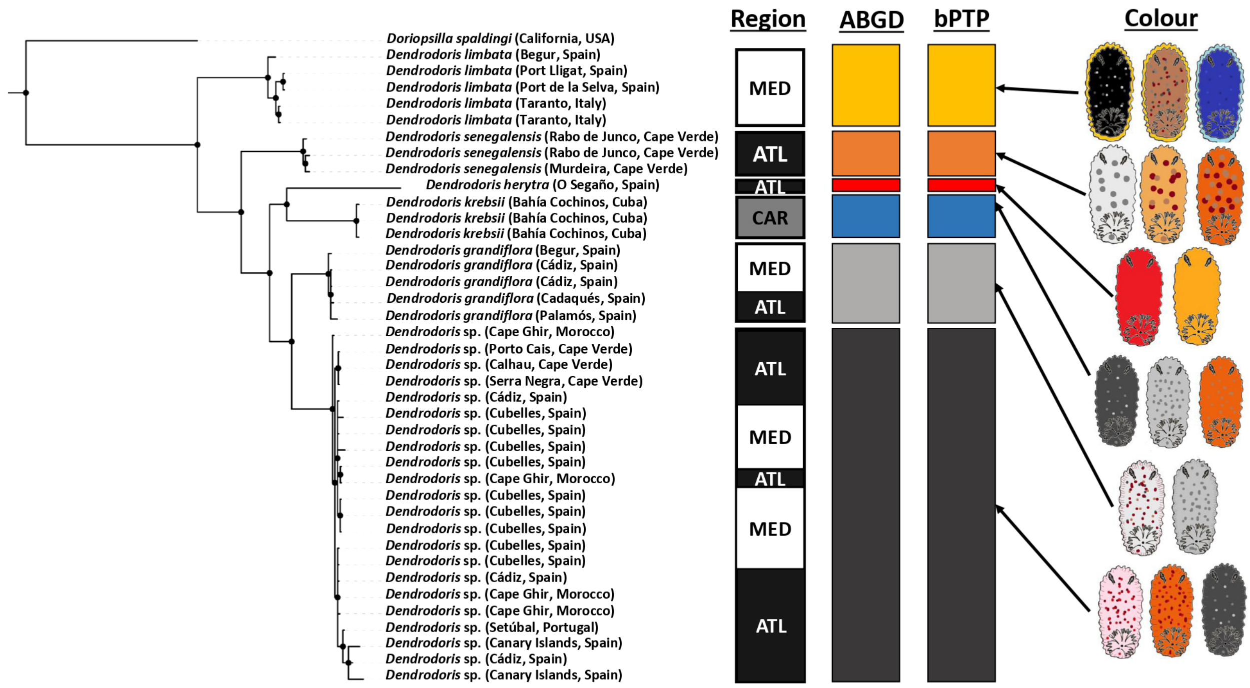


FIGURE 2. Bayesian phylogenetic tree obtained from the analysis of the COI marker, including the geographic region of each specimen (MED=Mediterranean, EATL= East Atlantic, WATL=West Atlantic), the ABGD and bPTP delimitation analyses results and schematic chromatic patterns of each species.

Species delimitation analysis. The ABGD analyses of the COI sequences with the three evolutionary models, as well as the bPTP analysis, recovered six candidate species (Fig. 2). The uncorrected p-distance values of the species show maximum intraspecific distance of a 3% in *Dendrodoris* sp. (Table 2). The minimum interspecific difference values between the *Dendrodoris* sp. and the other *Dendrodoris* species included in this study are 8% compared to *D. grandiflora*, 12% to *D. krebsii*, 13% to *D. senegalensis*, 15% to *D. herytra* and 16% to *D. limbata*.

The genetic differences described above, a morphological comparison with other species of *Dendrodoris*, and a review of the literature, leads us to identify *Dendrodoris* sp. as *Dendrodoris temarana* Pruvot-Fol, 1953, which until now had been considered synonymous with *D. grandiflora*. Below we provide a formal Redescription of *D. temarana*, recovering this species as valid. In addition, due to the inexistence of a holotype deposited in any biological institution, we designate here a neotype.

TABLE 2. Minimum and maximum COI gene pairwise uncorrected p-distances between *Dendrodoris* species included in the present study.

	Dendrodoris grandiflora	***Dendrodoris herytra***	***Dendrodoris krebsii***	***Dendrodoris limbata***	***Dendrodoris senegalensis***	***Dendrodoris temarana***
Dendrodoris grandiflora	0%–1%					
Dendrodoris herytra	15%–16%	-				
Dendrodoris krebsii	11%–13%	16%	0			
Dendrodoris limbata	14%–16%	20%–21%	15%–17%	0%–2%		
Dendrodoris senegalensis	11%–13%	17%–18%	14%–15%	16%–17%	1%	
Dendrodoris temarana	8%–10%	15%–17%	12%–15%	16%–19%	13%–15%	0%–3%

Systematics

Order Nudibranchia Cuvier, 1817

Suborder Doridina Odhner, 1934

Superfamily Phyllidioidea Rafinesque, 1814

Family Dendrodorididae O'Donoghue, 1924 (1864)

Genus *Dendrodoris* Ehrenberg, 1831

Type species. *Dendrodoris limbata* (Cuvier, 1804).

Dendrodoris temarana Pruvot-Fol 1953

Dendrodoris temarana Pruvot-Fol, 1953: 87–88, text fig. 31, pl. 3, fig. 55.
Dendrodoris limbata [non Cuvier (1804)]—Ballesteros (1978, 1984, 1985), García-Gómez (1984), Gómez-Álvarez (2017)
Dendrodoris grandiflora [non Rapp (1827)]—Valdés *et al.* (2016), Cyrne *et al.* (2018)
Dendrodoris herytra [non Valdés & Ortea in Valdés, Ortea, C. Ávila & Ballesteros (1996) in part] – Wirtz (1998), Cyrne *et al.* (2018)

Type material. The location of the holotype of *Dendrodoris temarana* is unknown, since the material examined by Pruvot-Fol (1953) was not deposited in any institution (P. Bouchet, pers. comm). One specimen collected from the type locality is designated as neotype herein.

Neotype. (Fig. 3A). Temara, Rabat-Salé-Kénitra, Morocco, (34º00'08.1"N 6º52'36.3"W) 30/03/2010, one specimen of 20 mm alive (pending number MNCN-15.05/200177; sequenced) col N. Tamsouri.

Additional material. Cape Ghir, Sus-Masa, Morocco, 27/04/2010 1 specimen 7 mm length preserved (pending number MNCN-15.05/94684; sequenced); 08/10/2009, one specimen 15 mm length alive (pending number MNCN-15.05/94683; sequenced); 22/06/2009, one specimen 8 mm length preserved (pending number MNCN-15.05/94682; sequenced), leg. N. Tamsouri; Cubelles, Catalonia, Spain, 05/04/2015, 1 specimen 17 mm length preserved (CRBA-92726; dissected); 30/06/2015, one specimen 40 mm length alive (CRBA-92721; sequenced) (Fig 3 C, D); one specimen 12 mm preserved (CRBA-92742; dissected and sequenced); 13/10/2015, two specimens 3 and 5 mm length preserved (CRBA-92730; dissected); six specimens 4, 4, 5, 5, 5 and 6 mm length (CRBA-92733; dissected); 13/11/2015, one specimen 5 mm length preserved (CRBA-92734; dissected); 14/12/2015, four specimens 7, 7, 8 and 11 mm length preserved (CRBA-92724; dissected); one specimen 37 mm length alive (CRBA-92737; sequenced) (Fig. 3E,F), one specimen 25 mm length alive (CRBA-92738; dissected and sequenced) (Fig. 3G,H); one specimen 19 mm length alive (CRBA-92741; sequenced); one specimen 38 mm length alive (CRBA-92743; sequenced); 15/02/2016, one specimen 33 mm length alive (CRBA-92744; sequenced); 14/03/2016, one specimen 12 mm length preserved (CRBA-92732; dissected); one specimen 25 mm length alive (CRBA-92739; sequenced); one specimen 35 mm length alive (CRBA-92740; sequenced); 18/04/2016, two specimens 17 and 27 mm length preserved (CRBA-92728; dissected), leg. C. Galià-Camps & M. Ballesteros; 29/10/2020, one specimen 18 mm length alive (SMF 360682) leg. M. Ballesteros; La Caleta, Cádiz, Andalusia, Spain, 02/11/2017, one specimen 23 mm length preserved (CRBA-92747; sequenced); one specimen 20 mm length preserved (CRBA-92735, sequenced); one specimen 17 mm length preserved (CRBA-92736, sequenced), leg. C. Galià-Camps; Gran Canaria, Canary Islands, Spain, 25/01/2017 one specimen 15 mm length preserved (CRBA-92745; sequenced); one specimen 14 mm length preserved (CRBA-92746; sequenced), leg. A. Herrero; Mitrena, Setúbal, Portugal, 20/04/2017, one specimen 26 mm length preserved (CRBA-92748; sequenced); Tróia, Setúbal, Portugal, 20/04/2017, one specimen 27 mm length preserved (CRBA-92749; sequenced), leg. G. Calado; Calhau, São Vicente, Cape Verde, 10/07/2013, one specimen 25 mm length preserved (MNCN-15.05/200125, sequenced), leg. M. Pola & J. Reis. Serra Negra, Sal, Cape Verde, 05/04/2014, one specimen 9 mm length preserved (MNCN-15.05/92207, sequenced); Porto Cais, Maio, Cape Verde, 05/04/2007, one specimen 12 mm length preserved (MNCN-15.05/92208, sequenced); Estancinha, Boavista, Cape Verde, 04/2019, one specimen 10 mm length preserved (MNCN-15.05/92209, sequenced), leg. M. Jiménez & C.M. Alfonso.

External morphology. Live specimens up to 70 mm in length. Body elongated, posterior part of foot protrudes slightly behind mantle. Mantle soft without spicules, very wide relative to foot, smooth and wavy, slightly striated, generally lighter than rest of body. Juvenile specimens uniformly pale red, shifting to adulthood (Fig. S5). Adults with variable chromatic pattern; red, pinkish, yellowish, orange, dark brown or almost black (Figs. 3A, C, E, H). Uniform background color (usually in black or brown specimens) or with orange, reddish and brownish spots. Rhinophores with 15–20 oblique lamellae, same color as body, tips white (Fig. 3G). Rhinophoral sheaths with smooth edge, slightly raised above mantle. Gill with eight branchial leaves, two most posterior with common base (Figs. 3D, F). Rare cases of six (juveniles of 5–10mm), or 10 (largest specimens) branchial leaves observed. Gill with same color as mantle, sometimes darker, with lighter rachis, white apices. Anal papilla somewhat globose and prominent, with upper edge lobed. Ventrally, mantle margin, body flanks and foot with same color as the dorsum, lacking spots.

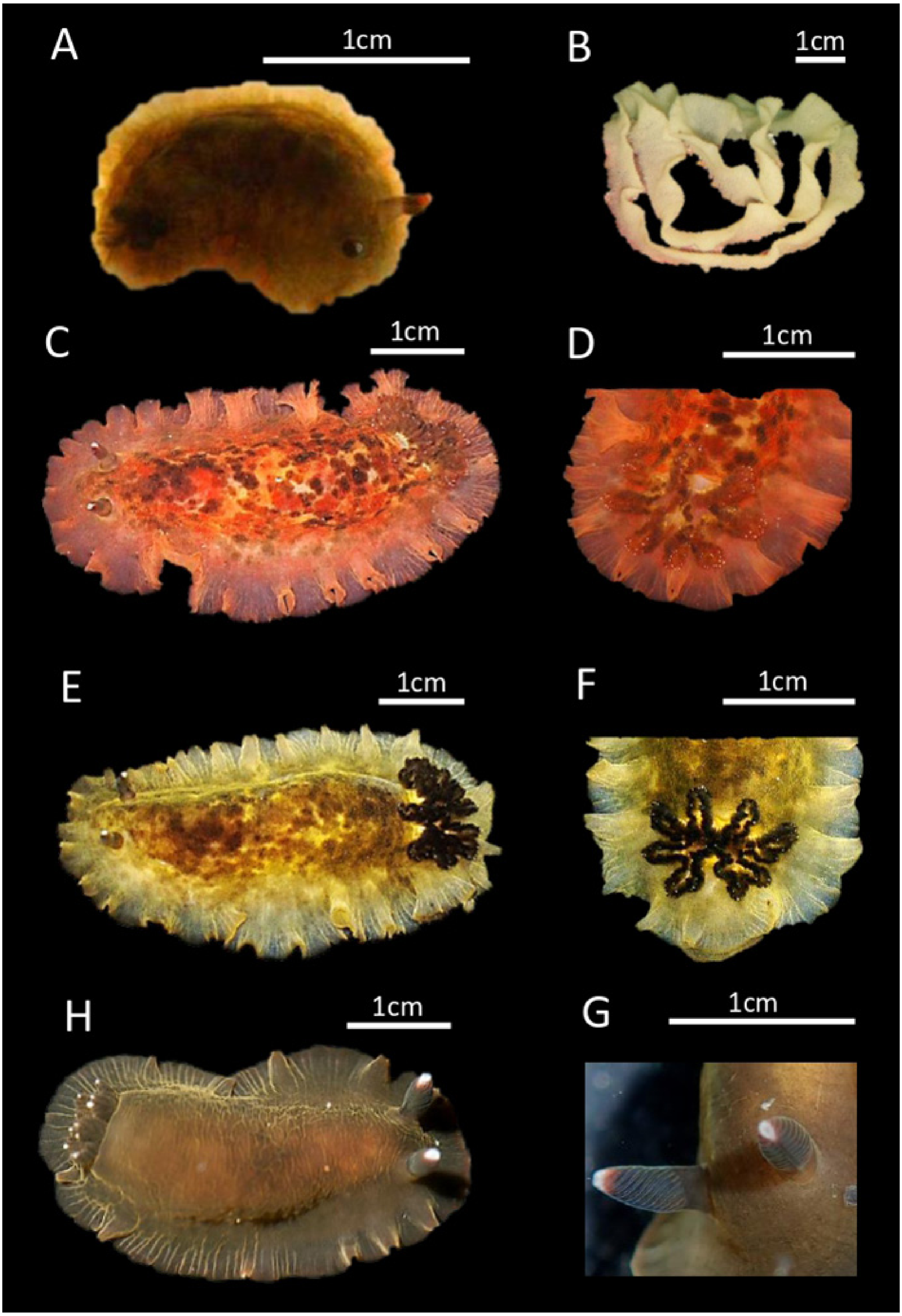


FIGURE 3. Chromatic variation in *Dendrodoris temarana*. **A**. **Neotype** MNCN-15.05/200177 (Temara). **B**. Egg mass. **C–D**. Specimen CRBA-92721 (Cubelles) with detail of the gill leaves. **E–F**. Specimen CRBA-92737 (Cubelles) with detail of the gill leaves. **G–H**. Specimen CRBA-92738 (Cubelles) with detail of the rhinophores.

Internal anatomy. Buccal bulb small in relation to size of animal, surrounded by thin membrane with black dots. Esophagus with two folds (Figs. 4A, B). Intestine with numerous loops after emerging from the visceral mass proximally, becoming visible again between lobes of digestive gland (Fig. 4A, B). Ptyalin gland yellowish, located below folds of esophagus; highly developed and divided into two main lobes, composed of smaller lobes (Fig. 5A, B). Ptyalin gland joined to buccal bulb, anterior to nervous esophageal collar. Digestive gland brown, lobulated, occupying posterior half of visceral mass. A lobe of digestive gland located posterior to reproductive system (Fig. 4B). Central nervous system posterior to buccal bulb. Heart, surrounded by pericardial membrane, located on posterior lobe of digestive gland. Aorta directed forward, running over organs of visceral mass. Blood gland brown, left of anterior region of aorta (Figs. 4A, B). Hermaphroditic gland granular, lobed, yellowish, occupying central part of visceral mass (Fig. 5C, D). Rest of reproductive system in anterior right area of visceral mass. Female gland complex well developed with distinct mucus gland posteriorly. Ampulla folded with at least three folds under prostate. Bursa copulatrix between ampulla and prostate, 3–4 times larger than seminal receptacle. Prostate long, thick, granular, profusely folded on itself. Vas deferens with sinuous proximal portion, straight distally. Penial hooks small, with elongated base seen by transparency (Fig. 6).

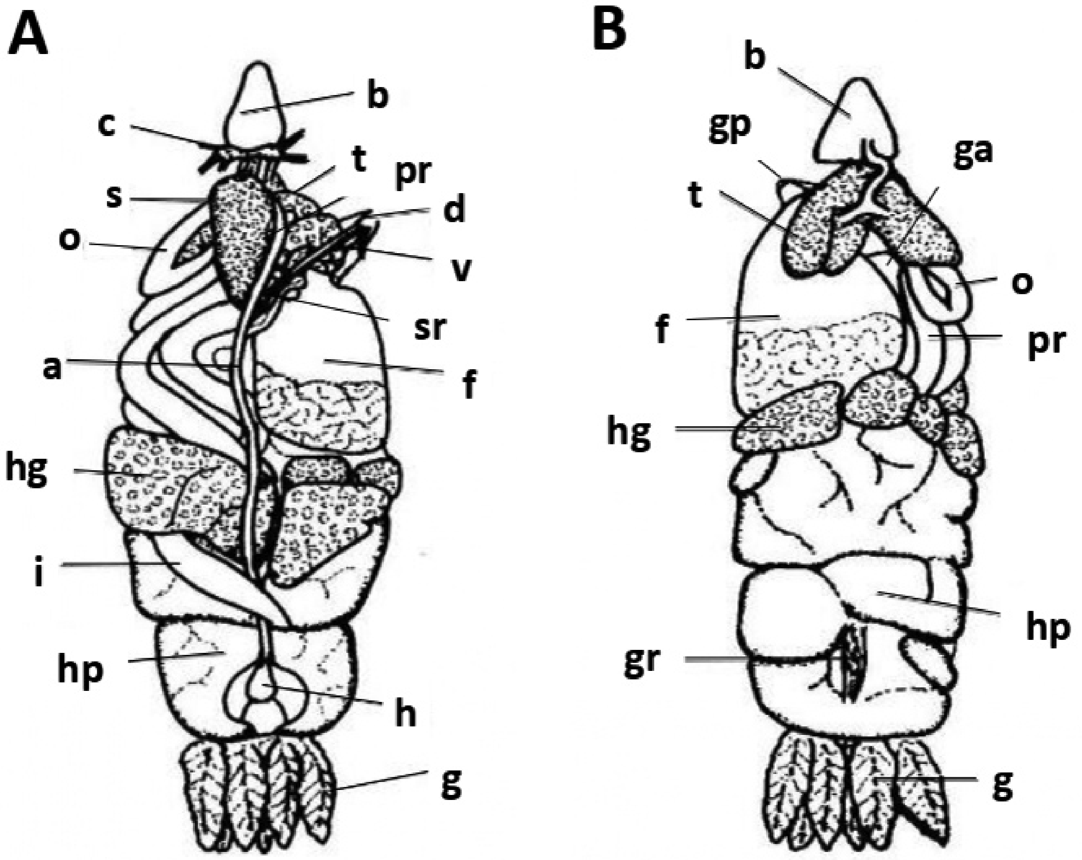


FIGURE 4. Internal anatomy of a 27 mm preserved, mature specimen of *Dendrodoris temarana* (CRBA-92728). **A**. Dorsal view. **B**. Ventral views. Abbreviations: ampulla; b: pharyngeal bulb; c: central nervous system; d: deferent duct; f: female gland; g: gill; ga: bursa copulatrix gp: genital pore; h: heart; hg: hermaphrodite gland; hp: digestive gland; i: intestine; o: esophagus; pr: prostate; s: blood gland; sr: seminal receptacle; t: ptyalin gland; v: vagina.

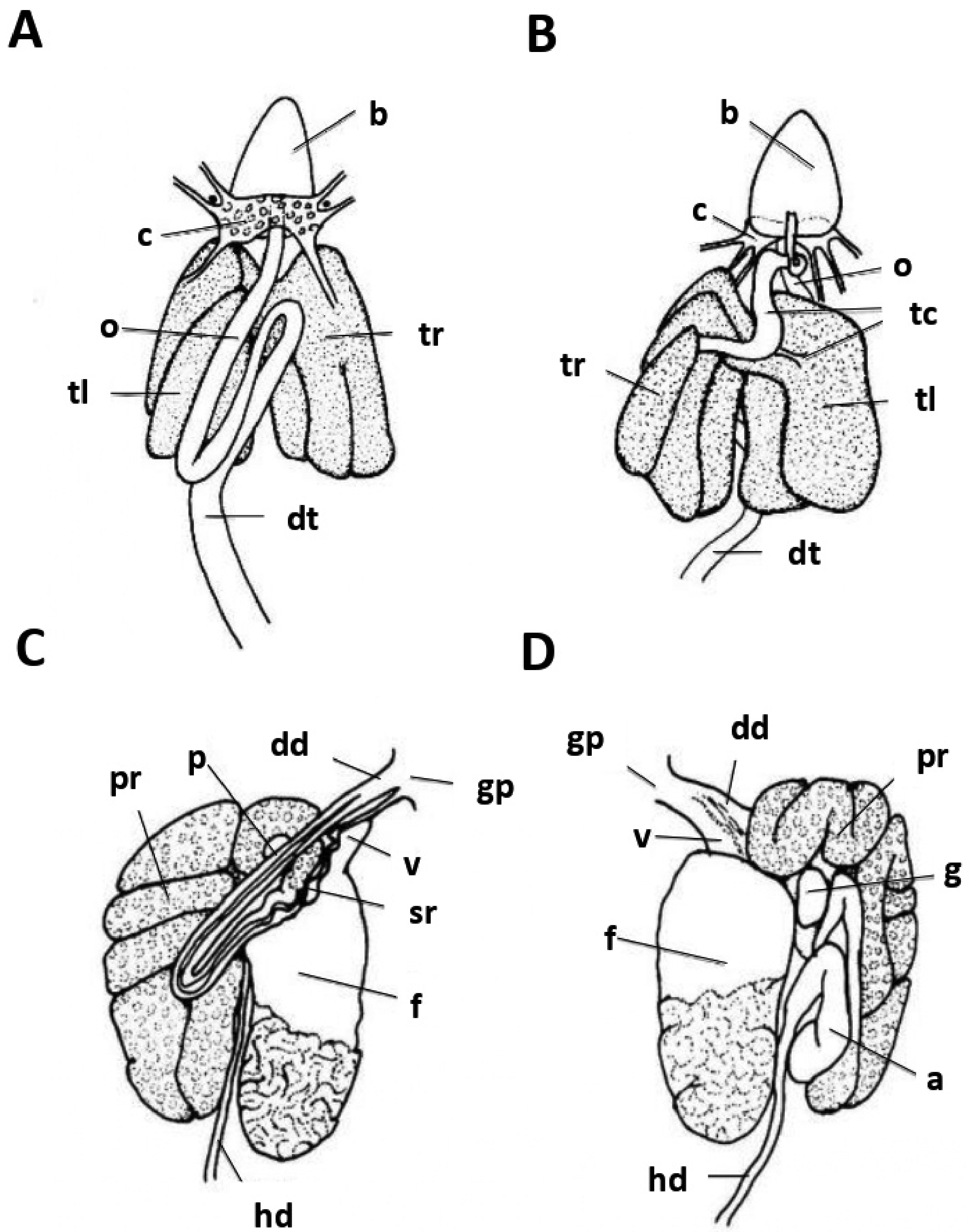


FIGURE 5. Anterior region of a 27 mm preserved *Dendrodoris temarana* (CRBA-92728). Digestive system. **A.** Dorsal view. **B.** Ventral view. Reproductive system. **C.** Dorsal view. D. Ventral view. Abbreviations: a: ampulla; b: pharyngeal bulb; bc: bursa copulatrix; c: central nervous system; dd: deferent duct dt: digestive tube; f: female gland; hd: hermaphroditic duct; o: esophagus; p: penis; pr: prostate; sr: seminal receptacle; tc: ptyalin duct; tl: left ptyalin gland; tr: right ptyalin gland; v: vagina.

Habitat and biology. *Dendrodoris temarana* is found under stones from the intertidal zone to several meters deep. In Cubelles, Catalonia, the species is common and has been documented for forty years in shallow waters (0–1 m deep) under boulders. Groups of 3–5 specimens can be found gathering under the same stone. Visual censuses carried out in the 1980s reported up to 40 specimens per hour of sampling (MB pers. obs.). In Cádiz, *D. temarana* is the most abundant nudibranch, co-occurring with *D. grandiflora*, much less abundant, under exposed stones at low tide (pers. obs.). Egg masses are laid under stones and are 8 mm wide, ivory-colored ribbons, coiled into a spiral with several whorls, which can reach 5 cm in total diameter (Fig. 3B). There are usually 60–70 eggs across the width of the ribbon, arranged in two superposed layers. The eggs have a diameter of about 80 µm and are enclosed in larger capsules of approximately 140 µm in diameter. Egg laying has been observed from May to November (JLC, MB, pers. obs.).

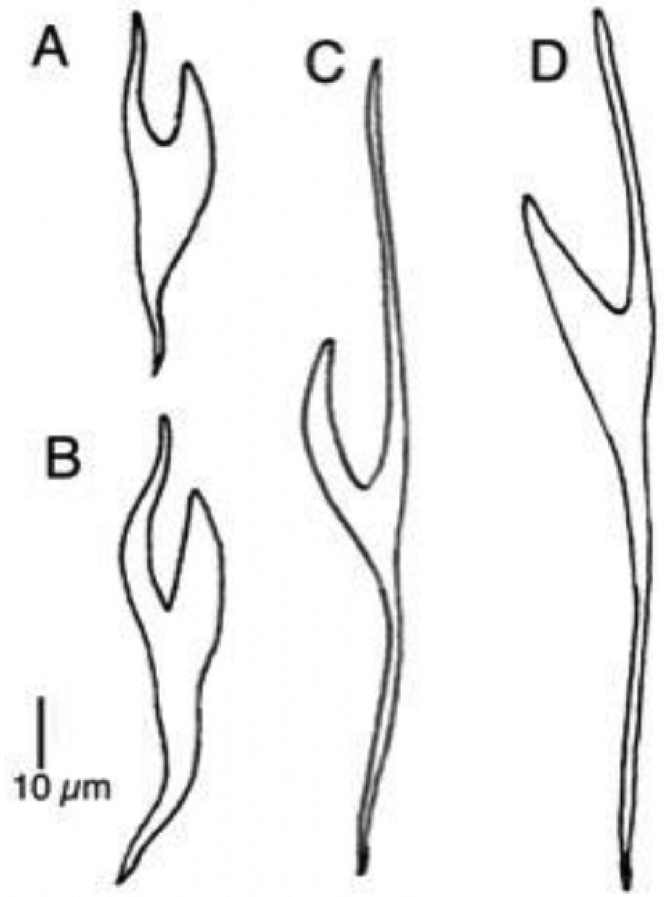


FIGURE 6. *Dendrodoris temarana* penial hooks.

Geographic distribution. *Dendrodoris temarana* ranges from western Mediterranean Sea, South Portugal, Morocco, Canary Islands and Cape Verde (Fig. 7). In Catalonian coast (NE Spain) and Cádiz (Andalusia, SW Spain) it is very abundant. Recorded from El Portil (SW Spain) (García-Gómez 1984), Gulf of Cádiz (Gómez-Álvarez 2017) and Cubelles (Ballesteros 1978, 1984, 1985) as *D. limbata*. Records from the Portugese mainland (Cyrne *et al.* 2018), the Azores (Wirtz 1995, 1998) and the Canary Islands (Valdés *et al.* 1996) were misidentified as *D. herytra*. This species was also misidentified as *D. grandiflora* in Portugal (Cyrne *et al.* 2018), the Canary Islands (Valdés *et al.,* 1996), Agadir (Valdés *et al.* 1996), and Mauritania (Valdés *et al.* 1996). Photographs or video records corresponding to *Dendrodoris temarana* from Thau (SE France), Ebro Delta (NE Spain), the Balearic Islands (E Spain), and La Línea de la Concepción (S Spain) are available from various internet websites (Suppl. Table 1).

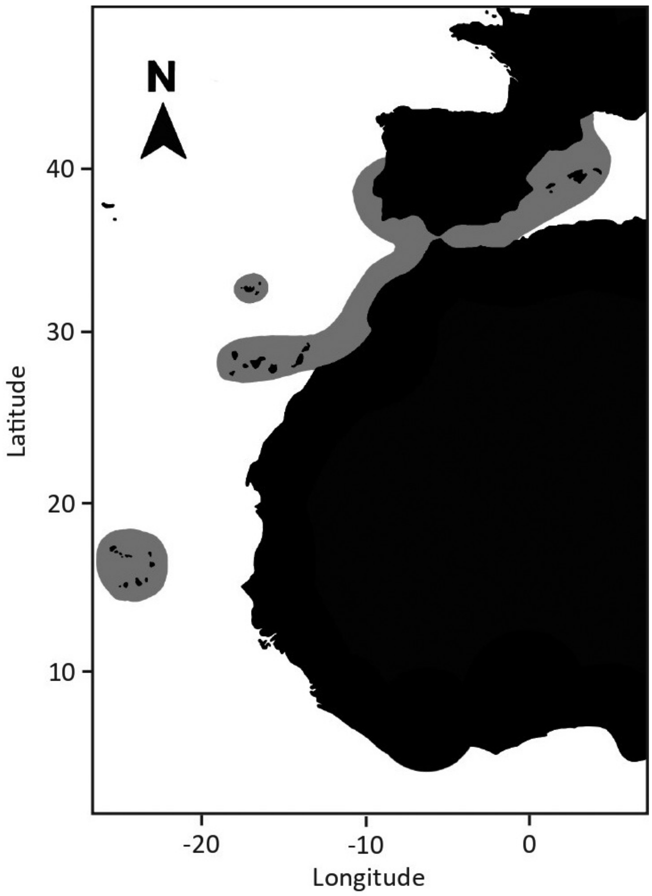


FIGURE 7. Geographic distribution of *Dendrodoris temarana*.

Remarks. *Dendrodoris temarana* was described by Pruvot-Fol (1953) based on six specimens collected by Hélène Gantés and Jean B. Panouse in the town of Temara, on the Atlantic coast of Morocco. The only illustration of the living specimens of the original description is a color drawing made by Gantés which appears in Pruvot-Fol (1953, pl. 3, fig 55). Thanks to this drawing and the original description of the internal morphology we have been able to recognize the unidentified Atlantic-Mediterranean specimens recovered in the phylogenetic and species delimitation analyses as *D. temarana*. Since the original type material was never deposited (Bouchet, Gofas, pers. com.) and its location is unknow, a neotype collected in Temara (Morocco) is hereby designated to fix the taxonomic status of this species, which is often confused with *Dendrodoris grandiflora* as well as with *Dendrodoris herytra* and *Dendrodoris limbata*. The designation of a neotype is therefore consistent with Article 75 of the ICZN (1999) as there is an exceptional need to clarify the status of this species. The neotype closely resembles the external morphology of the original description by Pruvot-Fol (1953, pl. 3, fig 55) (Fig. 3).

Dendrodoris temarana can be distinguished from the other *Dendrodoris* species from the Atlantic and Mediterranean Sea by its chromatic pattern, since it is the only species that has bright colorful background patterns ranging from yellow to reddish (Fig 3A, C, E) or even black (Fig 3H), as observed by Pruvot-Fol (1953) in some of its preserved specimens, as well as the absence of patches on the foot and internal differences in the digestive and reproductive systems. *Dendrodoris temarana* has been misidentified as *D. grandiflora* (Valdés *et al*., 1996) and *D. limbata* (Ballesteros 1978), however, in *D. grandiflora* the dorsum is pale grey with brownish and black patches (Valdés *et al*., 1996. fig 1C), with some spots on the lateral side of the foot, while the dorsum of the dark morphotype of *D. temarana* has no patches and the foot is totally patchless (Table 3). Specimens of *D. limbata* can have colorful backgrounds, with well-delineated dark dorsal patches and a clear yellow-orange wide band on the perimeter of the body (Valdés *et al*., 1996. fig 1A, B). *Dendrodoris temarana* can be distinguished from *D. limbata* because it has a brighter color pattern, diffuse patches on the dorsum and lacks the yellow margin (Table 3). Moreover, specimens of *D. limbata* have numerous dark spots on the foot and the flanks of the body ventrally, while specimens of *D.*

temarana have a uniform ventral color, without spots. When compared to NE-Atlantic *Dendrodoris* species, *D. temarana* is easily distinguishable from *D. herytra*, which has a uniform orange to reddish notum without patches and a small gill. Black colored *D. herytra* individuals reported by Valdés *et al*. (1996) are misidentifications of *D. temarana*, which has a broad color spectrum.

TABLE 3. Comparison among the Atlantic and Mediterranean species of the genus *Dendrodoris* using main morphological characters based on Valdés *et al*., 1996 and current study observations.

Species	***Dendrodoris grandiflora***	***Dendrodoris herytra***	***Dendrodoris krebsii***	***Dendrodoris limbata***	***Dendrodoris senegalensis***	***Dendrodoris temarana***
Chromatic pattern	Pale grey background, usually with black spots on notum.	Bright reddish background without patches on notum	Bright. Variable ranging from white to black. Diffused spots on notum	Pale. Variable from yellow to black. Presence of well-defined patches on notum. Yellow margin	Bright. Variable ranging from white to black. Defined large spots on notum	Bright. Variable ranging from yellow to black. Presence of diffused spots on notum.
Foot color	Same as dorsum. Without or with few patches	--	--	Blackish. Several black patches	--	Same as body. Without patches.
Branchial leaves	Large	Small	Small	Large	Large	Large
Oesophagus	Three folds	Five folds	Four folds	Five folds	Three folds	Two folds
Penial hooks	Small with elongated bases	Small with some elongated bases	Small with elongated bases	Large with narrow bases	Small with some elongated bases	Small with elongated bases
Vas deferens	Straight	Four folds	One fold	Curved	Two folds	Straight
Ampulla	One fold	One fold	One fold	Four folds	Two folds	Three folds
Female gland	Small	Very small	Large	Massive	Large	Small
Egg mass color	Yellowish	--	Yellowish	Orange	Yellowish	Whitish
Eggs size	100 μm	--	66 μm	333–523 μm	--	80 μm
Egg capsule size	190 μm	--	85 μm	371–567 μm	110 μm	140 μm

Dendrodoris temarana possess several morphological similarities to *D. senegalensis*, a species with which is partially sympatric (Table 3). The only external trait which may distinguish *D. temarana* and *D. senegalensis* is the size of the dorsal patches, larger in *D. senegalensis*. Nevertheless, internal anatomy features are much more reliable to differentiate these two species since *D. temarana* has two folds in the esophagus (Fig. 5A) and *D. senegalensis* has three of them (Valdés *et al*. 1996, fig 9). Moreover, their reproductive systems are clearly different, with *D. temarana* having a female gland as large as the prostate and three folds in the ampulla (Figs. 5C–D), while the female gland of *D. senegalensis* is 3–4 times as large as the prostate and the ampulla has two folds (Valdés *et al.* 1996, fig 9). Also, the deferent duct and vagina of *D. senegalensis* are folded several times (Valdés *et al*., op. cit.), while they are long and straight with a fold at the proximal end in *D. temarana*. The shape of the penial hooks is also different, having elongated bases in all of the hooks of *D. temarana* (Fig. 6), while this is not the case in *D. senegalensis* (Valdés *et al*., 1996, fig 9). The egg masses are also different, white in *D. temarana* (Fig. 3B) and yellow and narrow in *D. senegalensis* (Table 3). Finally, *D. temarana* is morphologically similar to the Caribbean species *D. krebsii*. However, these two species can be externally distinguished by the gill size, large in *D. temarana* but smaller in *D. krebsii*. Internally, *D. krebsii* differs from *D. temarana* because its esophagus has four folds, the ampulla has only one-fold, and the vagina and vas deferens have at least two folds (Valdés *et al* 1996, fig 7); moreover, the egg mass of *D. krebsii* is yellow, with small egg capsules (85 μm) (Valdés *et al*. 1996) compared to those of *D. temarana* (140 μm) (Table 3).

Two other species described by Pruvot-Fol, *Dendrodoris pseudorubra* Pruvot-Fol, 1951 and *Dendrodoris longula* Pruvot-Fol, 1951 are possible synonyms of *D. temarana*. However, due to the lack of information in these descriptions, we are unable to confirm their identity, and therefore we consider *Dendrodoris temarana* as the valid species name.

Discussion

The data here presented is more than sufficient to achieve the main objective of this paper, which is to clarify the taxonomic status of Atlantic-Mediterranean species by using molecular methods. Moreover, these data have allowed us to redescribe the species *Dendrodoris temarana* as valid and distinct from other species in the region, as it was here recovered as a monophyletic clade. All mitochondrial phylogenetic analyses support the monophyly of the previously accepted species and the newly reinstated *D. temarana*. This topology is also supported in the analyses with the concatenated COI+16S and COI+16S+H3 datasets. Additional support for the distinctiveness of the Atlantic-Mediterranean species is provided by the uncorrected p-distances, which show a large percentage of substitutions in comparison to each intraspecific species value (Table 2), as well as the results of the species delimitation analyses ABGD and bPTP (Fig. 2), both of which recover *D. temarana* as a separate species.

Dendrodoris temarana is extremely variable in color pattern but otherwise similar morphologically to the other North Atlantic and Mediterranean *Dendrodoris* species, and for this reason it has been repeatedly misidentified in the literature as *D. limbata* (Ballesteros 1978, 1984, 1985), *D. grandiflora* and *D. herytra* (Valdés *et al*. 1996, Cyrne *et al*. 2018). While the taxonomic reliability of color pattern is questioned due to the high variability within and between species of a given group (Goodheart *et al.* 2015; Padula *et al.* 2016; Layton et al. 2018; Galià-Camps *et al.* 2020), the usefulness of color traits to differentiate species has been recently demonstrated in other marine heterobranch sea slug genera such as *Trinchesia* (Korshunova *et al*. 2017), *Placida* (McCarthy *et al*. 2019) and *Elysia* (Martín-Hervás *et al*. 2020). Data here presented reveal that color patterns are consistent enough to play an important role defining *Dendrodoris* species from the Mediterranean Sea and adjacent Atlantic Ocean (Fig. 2, Table 3), and provide additional support for the validity of *D. temarana*.

Although the analyses here presented have enough resolution and provide support for most clades, two node values are unsupported when using ML methodologies, there is a mito-nuclear discordance in the markers used—also reported in Hallas *et al.* (2017) and Korshunova *et al.* (2020)—and there are alignment difficulties when aligning COI and 16S with the outgroup species. To fully clarify the relationships among species of *Dendrodoris*, additional analyses using genomic methodologies may be necessary, as well as a more comprehensive taxon sampling including the Atlantic-Mediterranean species missing from this study. Nevertheless, based on the evidence here provided, COI is an adequate molecular barcode marker for the species of the genus *Dendrodoris* in the study area. This is based on the fact that all COI sequences obtained are clearly orthologs and align properly. Thus, we encourage future studies using this molecular marker to explore the biodiversity of this genus, which may help to identify additional cryptic species.

Acknowledgements

This work was supported by the Generalitat de Catalunya (SGR2017-1120), the Fundación Biodiversidad of the Ministerio Español de Transición Ecológica (Project BIOCALETA), the Ministerio Español de Educación y Ciencia (CGL2006-05182/BOS), the Ministerio Español de Ciencia e Innovación (CGL2010-17187) and the Universidad de Cádiz (PR2018-039). The authors want to thank to the sample providers Naoufal Tamsouri, Aketza Herrero, Victoriano Urgorri and Fabio Vitale. Also, to Oriol Castells, Josep Roma, Marta Pola, Joaquim Reis, Manuel J. Tenorio, Carlos Manuel Afonso and Peter Wirtz for their help in the sampling campaigns, to Alba Enguídanos and Leila Carmona for their efforts on the molecular lab and Miquel Pontes for his work searching for non-published reports of *D. temarana* on social network webpages. CG was supported by a PhD scholarship funded by the Ministerio Español de Ciencia, Innovación y Universidades (PRE2018-085227).

References

Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M.V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C.B., Brandão, S.N., Bray, R.A., Bruce, N.L., Cairns, S.D., Chan, T., Cheng, L., Collins, A.G., Cribb, T., Curini-Galletti, M., Gordon, D.P., Guiry, M.D., Hernandez, F., Hoeksema, B.W., Hopcroft, R.R., Jaume, D., Kirk, P., Koedam, N., Koenemann, S., Kolb, J.B., Kristensen, R.M., Kroh, A., Lambert, G., Lazarus, D.B., Lamaitre, R., Longshaw, M., Lowry, J., Macpherson, E., Madin, L.P., Mah, C., Mapstone, G., McLaughlin, P.A., Mees, J., Meland, K., Messing, C.G., Mills, C.E., Molodtsova, T.N., Mooi, R., Nehaus, B., Ng, P.K., Nielsen, C., Norenburg, J., Opresko, D.M., Osawa, M., Paulay, G., Perrin, W., Pilger, J.F., Poore, G.C., Pugh, P., Read, G.B., Reimer, J.D., Rius, M., Roch, R.M., Saiz-Salinas, J.I., Scarabino, V., Schierwater, B., Schmidt-Rhaesa, A., Schnabel, K.E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H., Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M.L., Theusen, E.V., Timm, T., Todaro, M.A., Turon, X., Tyler, S., Uetz, P., van der Land, J., Vanhoorne, B., van Ofwegen, L.P., van Soest, R.W., Vanaverbeke, J., Walker-Smith, G., Walter, T.C., Warren, A., Williams, G.C., Wilson, S.P., Costello, M.J. (2012) The magnitude of global marine species diversity. *Current Biology*, 22, 1–14.
https://doi.org/10.1016/j.cub.2012.09.036

Araujo, A.K., Pola, M., Malaquias, M.A.E. & Cervera, J.L. (2019) To be or not to be? What molecules say about *Runcina brenkoae* Thompson, 1980 (Gastropoda: Heterobranchia: Runcinida). *Scientia marina*, 83 (3), 223–235.
https://doi.org/10.3989/scimar.04907.07A

Araujo, A.K., Pola, M., Malaquias, M.A.E., Ballesteros, M., Vitale, F. & Cervera, J.L. (2021) 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*. *Zoological Journal of the Linnean Society*, 194, 761–788.
https://doi.org/10.1093/zoolinnean/zlab041

Ballesteros, M. (1978) Contribución al conocimiento de la fauna bentónica de Cubellas. *Publicaciones del Departamento de Zoología*, III, 11–23.

Ballesteros, M. (1984) Adición a la fauna de Opistobranquios de Cubellas. *Miscelánea Zoológica*, VIII, 41–49.

Ballesteros, M. (1985) Contribución al conocimiento de los Ascoglosos y Nudibranquios. *Publicaciones Ediciones Universidad de Barcelona*, 1985, 1–46.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22 (3), 148–155.
https://doi.org/10.1016/j.tree.2006.11.004

Bouchet, P. (1975) Nudibranches nouveaux des côtes du Senegal. *Vie et Milieu*, 25, 119–132.

Brodie, G. & Calado, G. (2006) *Dendrodoris arborescens* (Collingwood, 1881) (Mollusca: Nudibranchia): larval characteristics reveal a masked porostome species. *Records of the Western Australian Museum* Supplement No. 69, 119–126.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. & Gray, M.R. (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, 46, 419–437.
https://doi.org/10.1071/ZO98048

Cuvier, G. (1804) Mémorie sur le genre Doris. *Annales du Muséum National d'Historie Naturelle, Paris*, 4, 447–473.

Cyrne, R., Rosa, I.C., Faleiro, F., Dionísio, G., Baptista, M., Couto, A., Pola, M. & Rosa, R. (2018) Nudibranchs out of water: Long-term temporal variations in the abundance of two *Dendrodoris* species under emersion. *Helgoland Marine Research*, 72 (14). [published online]
https://doi.org/10.1186/s10152-018-0516-4

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2015) jModelTest 2: more models, new heuristics and high- performance computing. *Nature Methods*, 9 (8), 772.
https://doi.org/10.1038/nmeth.2109

Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32 (5), 1792–1797.
https://doi.org/10.1093/nar/gkh340

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 (5), 294–299.

Galià-Camps, C., Carmona, L., Cabrito, A. & Ballesteros, M. (2020) Double trouble. A cryptic first record of *Berghia marinae* Carmona, Pola, Gosliner, & Cervera 2014 in the Mediterranean Sea. *Mediterranean Marine Science*, 21 (1), 191–200.
https://doi.org/10.12681/mms.20026

García-Gómez, J.C. (1984) *Bulomorfos, ascoglosos y nudibranquios (Mollusca: Opisthobranchia) del estrecho de Gibraltar con algunas referencias al litoral onubense: estudio morfoanatómico, sistemático, faunístico y zoogeográfico*. Unpublished PhD Thesis, Universidad de Sevilla, Sevilla. [unknown pagination]

Goddard, J.H. (2005) Ametamorphic direct development in *Dendrodoris behrensi* (Nudibranchia: Dendrodorididae), with a review of developmental mode in the family. *Proceedings of the California Academy of Sciences*, Series 4, 56 (19), 201–211.

Golestani, H., Crocetta, F., Padula, V., Camacho-García, Y., Langeneck, J., Poursanidis, D., Pola, M., Yokeş, M.B., Cervera, J.L., Jung, D.-W., Gosliner, T.M., Araya F., Hooker, Y., Schrödl, M. & Valdés, Á. (2019). The little Aplysia's coming of age: From one species to a complex of species complexes in *Aplysia parvula* Mörch, 1863 (Mollusca, Gastropoda, Heterobranchia). *Zoological Journal of the Linnean Society*, 187 (2), 279–330. https://doi.org/10.1093/zoolinnean/zlz028

Gómez-Álvarez, G. (2017) *Guía de moluscos marinos de Huelva y del Golfo de Cádiz: con más de 250 espécies*. *Diputación Provincial de Huelv*a. Servicio de Publicaciones, Huelva, 300 pp.

Goodheart, J., Camacho-García, Y., Padula, V., Schrödl, M., Cervera, J.L., Gosliner, T.M. & Valdés, Á. (2015) Systematics and biogeography of *Pleurobranchus* Cuvier,1804 sea slugs (Mollusca: Gastropoda: Heterobranchia: Nudipleura: Pleurobranchidae). *Zoological Journal of the Linnean society*, 174, 322–362. https://doi.org/10.1111/zoj.12237

Hallas, J.M., Chichvarkhin, A. & Gosliner, T.M. (2017) Aligning evidence: concerns regarding multiple sequence alignments in estimating the phylogeny of the Nudibranchia suborder Doridina. *Royal Society Open Science*, 4 (10), 171095. https://doi.org/10.1098/rsos.171095

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

Hirose, M., Hirose, E. & Kiyomoto, M. (2015) Identification of five species of *Dendrodoris* (Mollusca: Nudibranchia) from Japan, using DNA barcode and larval characters. *Marine Biodiversity*, 45, 769–780.

Hoover, C., Lindsay, T., Goddard, J.H.R. & Valdés, A. (2015) Seeing double: pseudocryptic diversity in the *Doriopsilla albopunctata*-*Doriopsilla gemela* species complex of the north-eastern Pacific. *Zoological Scripta*, 44 (6), 612–631. https://doi.org/10.1111/zsc.12123

Huelsenbeck, J.P. & Rannala, B. (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology*, 53, 904–913. https://doi.org/10.1080/10635150490522629

Korshunova, T., Fletcher, K., Picton, B., Lundin, K., Kashio, S., Sanamyan, N., Sanamyan, K., Padula, V., Schrödl, M. & Martynov, A. (2020) The Emperor's Cadlina, hidden diversity and gill cavity evolution: new insights for the taxonomy and phylogeny of dorid nudibranchs (Mollusca: Gastropoda). *Zoological Journal of the Linnean Society*, 189 (3), 762–827. https://doi.org/10.1093/zoolinnean/zlz126

Korshunova, T., Martynov, A. & Picton, B. (2017) Ontogeny as an important part of integrative taxonomy in Tergipedid aeolidaceans (Gastropoda: Nudibranchia) with a description of a new genus and species from the Barents Sea. *Zootaxa*, 4324(1), 1–22. https://doi:10.11646/zootaxa.4324.1.1

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

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

Marcus, E. & Gallagher, S.B. (1976) A new species of *Dendrodoris* from Florida. *Veliger*, 18, 353–356.

Marsh, A.C., Ribbink, A.J. & Marsh, B.A. (1981) Sibling species complexes in sympatric populations of *Petrotilapia* Trewavas (Cichlidae, Lake Malawi). *Zoological Journal of the Linnean Society*, 71 (3), 253–264. https://doi.org/10.1111/j.1096-3642.1981.tb01132.x

Martín-Hervás, M.R., Carmona, L., Jensen, K.R., Licchelli, C., Vitale, F. & Cervera, J.L. (2020) Description of a new pseudocryptic species of *Elysia* Risso, 1818 (Heterobranchia, Sacoglossa) in the Mediterranean Sea. *Bulletin of Marine Science*, 96 (1), 127–143. https://doi.org/10.5343/bms.2018.0087

McCarthy, J.B., Krug, P.J. & Valdés, Á. (2019) Integrative systematics of *Placida cremoniana* (Trinchese, 1892) (Gastropoda, Heterobranchia, Sacoglossa) reveals multiple pseudocryptic species. *Marine Biodiversity*, 49, 357–371. https://doi.org/10.1007/s12526-017-0812-2

MolluscaBase (Eds.) (2022) MolluscaBase. *Dendrodoris* Ehrenberg, 1831. Accessed through: World Register of Marine Species. Available from: http://www.marinespecies.org/aphia.php?p=taxdetails&id=137883 (accessed 23 August 2021)

Mörch, O.A.L. (1863) Contributions à la faune malacologique des Antilles Danoises. *Journal de Conchyhology*, 11, 21–43.

Ortea, J. & Espinosa, J. (2001) Descripción de una nueva especie de *Dendrodoris* Ehrenberg, 1831. A new species of *Dendrodoris* Ehrenberg, 1831. *Avicennia*, (Supplement 4), 52–53.

Padula, V., Bahia, J., Stöger, I., Camacho-García, Y., Malaquías, M.A.E., Cervera, J.L. & Schrödl, M. (2016) A test of color-based taxonomy in nudibranchs: molecular phylogeny and species delimitation of *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., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (2002) *The Simple Fool's guide to PCR. Version 2.0*. Department of Zoology and Kewalo Marine Laboratory, Honolulu, 45 pp.

Park, J., Lee, Y., Shin, Y., Kim, T. & Park, J. (2019) *Dendrodoris guttata* (Nudibranchia: Dendrodorididae) from Korean Waters. *Animal Systematics, Evolution and Diversity*, 35 (1), 6–9. https://doi.org/10.5635/ASED.2019.35.1.039

Pfenninger, M. & Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7, 121.
https://doi.org/10.1186/1471-2148-7-121

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

Pruvot-Fol, A. (1951) Étude des Nudibranches de la Méditerranée. *Archives de Zoologie Experimentale et Generale*, 88,1–80.

Pruvot-Fol, A. (1953) Étude de quelques Opisthobranches de la Côte Atlantique du Maroc et du Sénégal. *Travaux de l´Institut Scientifique Chérifien*, 5, 1–93.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21, 1864–1877.
https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rambaut, A. (2014) *FigTree. Version 1.4.2.* Institute of Evolutionary Biology, The University of Edinburgh, Edinburgh. [software]

Rapp, W. (1827) Über das Molluskengeschlecht Doris und Beschreibung einiger neuen Arten desselben. *Nova Acta Physico-Medica Academiae Caesareae Leopoldino-Carolinae Naturae Curiosorum*, 13, 515–522.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12), 1572–1574.
https://doi.org/10.1093/bioinformatics/btg180

Rose, R.A. (1985) The spawn and embryonic development of colour variants of *Dendrodoris nigra* Stimpson (Mollusca: Nudibranchia). *Journal of the Malacological Society of Australia*, 7 (1–2), 75–88.
https://doi.org/10.1080/00852988.1985.10673977

Rüppell, E. & Leuckart, F.S. (1830) s.n .*In*: *Atlas zu der Reise im nordlichen Afrika van Eduard Rüppell. Zoology Neue wirbellose Thiere des Rothen Meers*. Gedruckt und in Commission bei Heinr. Ludw. Brönner, Frankfurt am Main, pp. 15–47.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a complitation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America*, 87 (6), 651–701.
https://doi.org/10.1093/aesa/87.6.651

Stamatakis A. (2014) RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Swindell, S.R. & Plasterer, T.N. (1997) SEQMAN. Contig assembly. *Methods in Molecular Biology*, 70, 75–89.

Valdés, A., Ortea, J.A., Ávila, C. & Ballesteros, M. (1996) Review of the genus *Dendrodoris* Ehrenberg, 1831 (Gastropoda: Nudibranchia) in the Atlantic Ocean. *Journal of Molluscan Studies*, 62 (1), 1–31.
https://doi.org/10.1093/mollus/62.1.1

Valdés, A. & Behrens, D.W. (1998) A new species of *Doriopsilla* (Mollusca, Nudibranchia, Dendrodorididae) from the Pacific coast of North America. *Proceedings-California Academy of Sciences*, 50, 307–314.

Valdés, Á. & Gosliner, T.M. (1999) Phylogeny of the radula-less dorids (Mollusca, Nudibranchia), with the description of a new genus and a new family. *Zoologica Scripta*, 28 (3–4), 315–360.
https://doi.org/10.1046/j.1463-6409.1999.00014.x

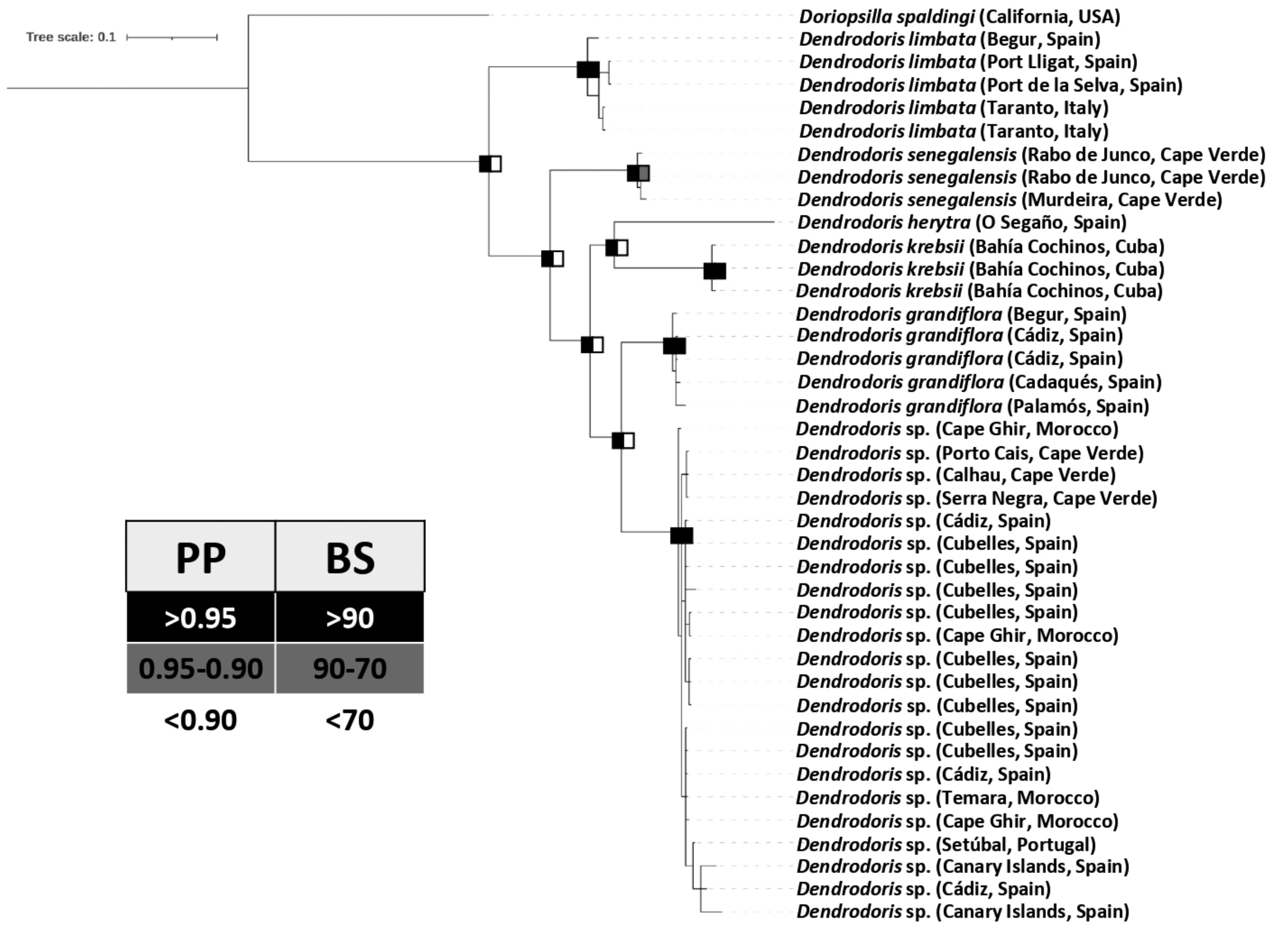
Wirtz, P. (1995) *Unterwasserführer Madeira, Kanaren/Azoren*. Delius Klasing, Edition Naglschmid. [unknown pagination]

Wirtz, P. (1998) Opisthobranch molluscs from the Azores. *Vita Marina*, 45, 1–16.

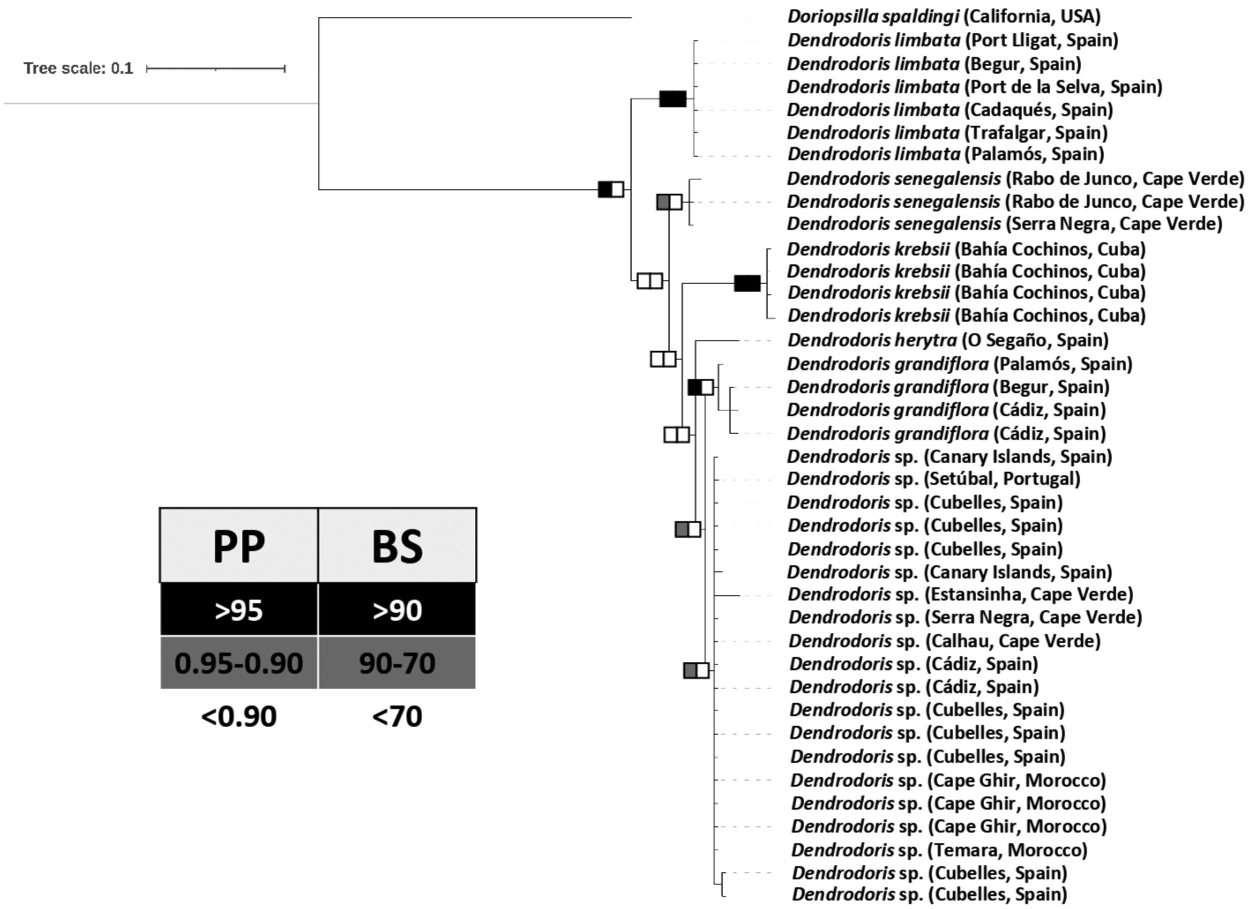
Zenetos, A., Çinar, M.E., Crocetta, F., Golani, D., Rosso, A., Servello, G., Shenkar, N., Turon, X., Verlaque, M. (2017) Uncertainties and validation of alien species catalogues: the Mediterranean as an example. *Estuarine, Coastal and Shelf Science*, 191, 171–187.
https://doi.org/10.1016/j.ecss.2017.03.031

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

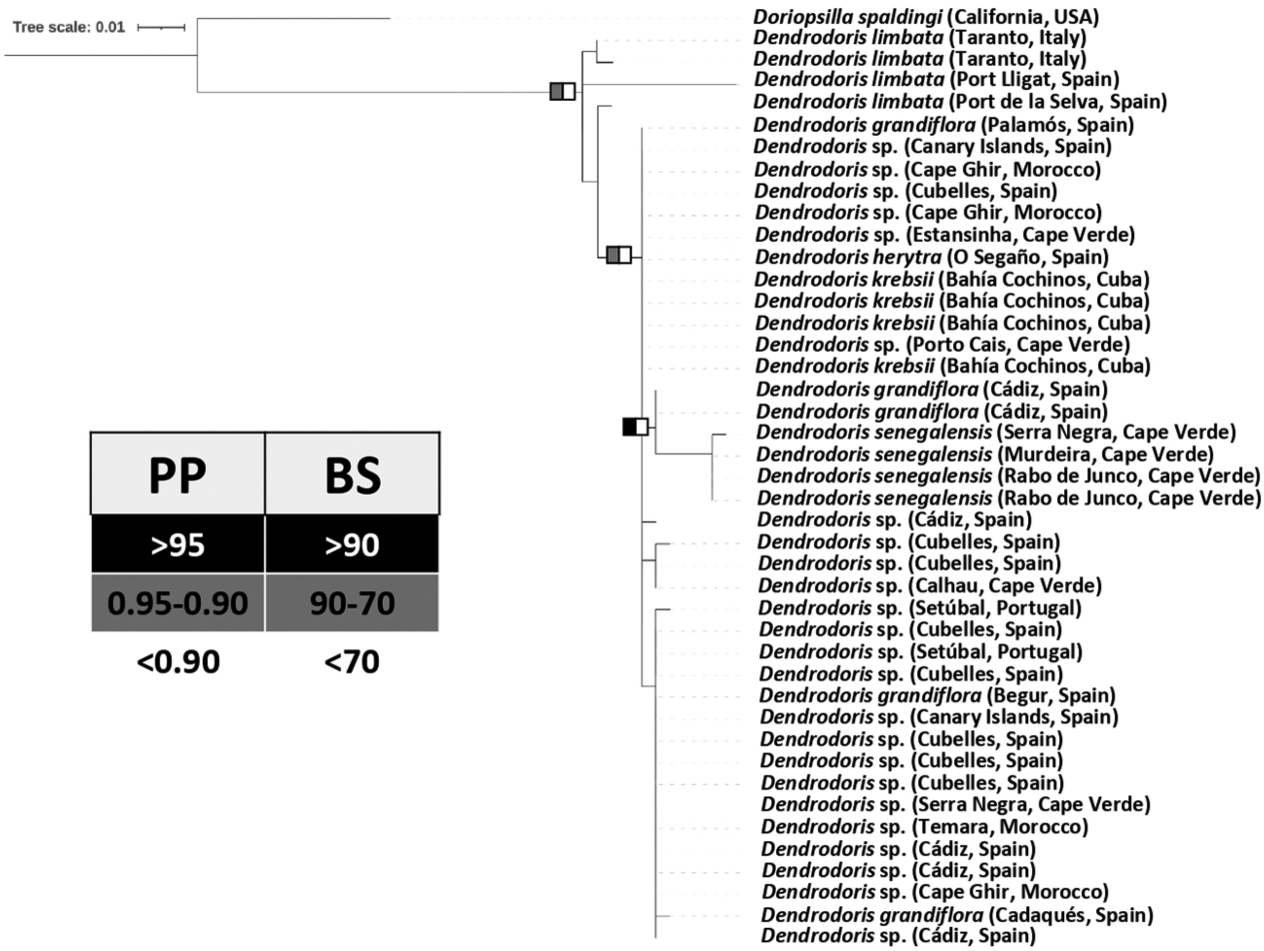
SUPPLEMENTARY FIGURE 1. Bayesian phylogenetic tree obtained from the analysis of the COI dataset. On nodes, first colored square represents PP values, and the second one BS values.



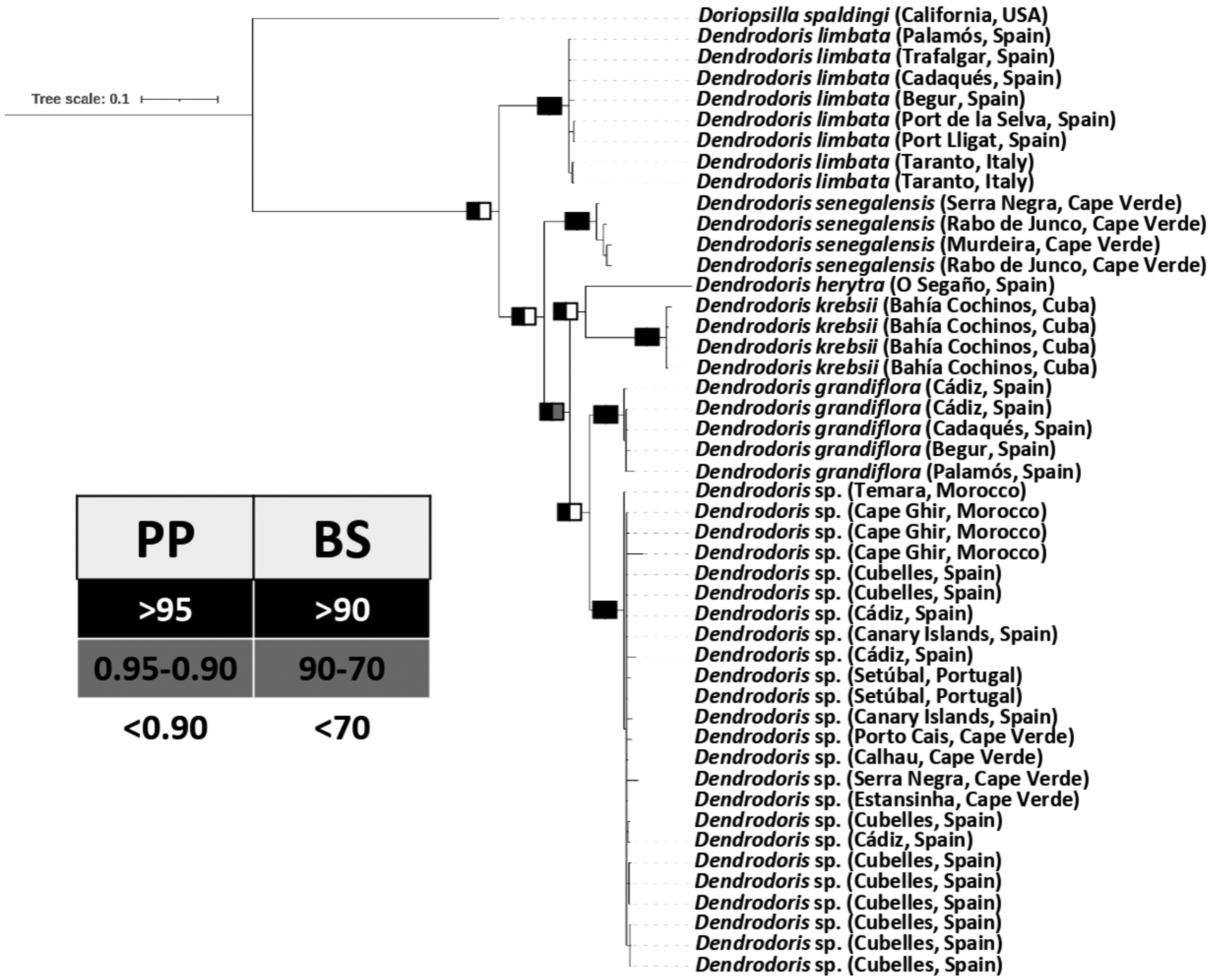
SUPPLEMENTARY FIGURE 2. Bayesian phylogenetic tree obtained from the analysis of the 16S dataset. On nodes, first colored square represents PP values, and the second one BS values.



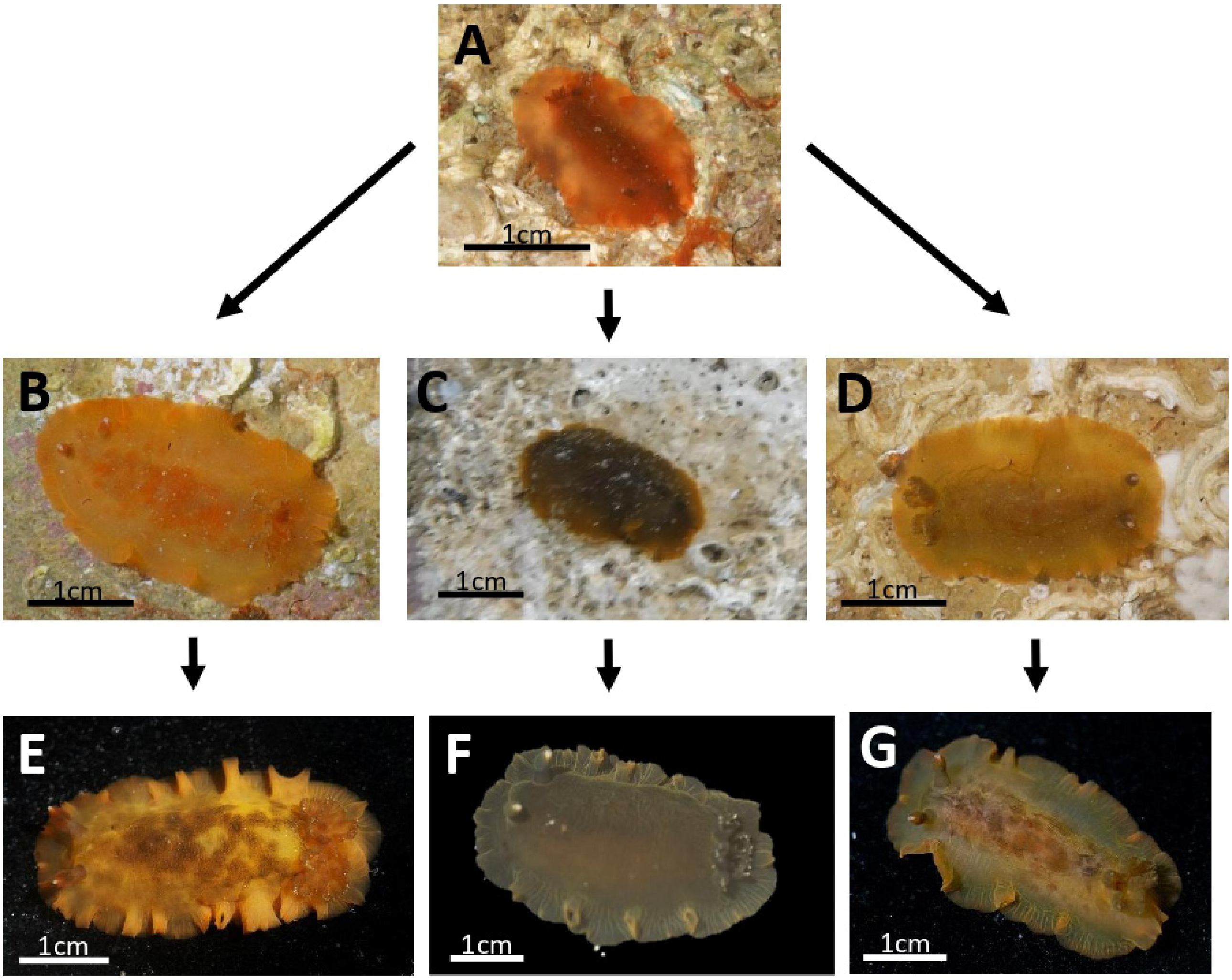
SUPPLEMENTARY FIGURE 3. Bayesian phylogenetic tree obtained from the analysis of the H3 dataset. On nodes, first colored square represents PP values, and the second one BS values.



SUPPLEMENTARY FIGURE 4. Bayesian phylogenetic tree obtained from the analysis of the COI+16S dataset. On nodes, first colored square represents PP values, and the second one BS values.



SUPPLEMENTARY FIGURE 5. Chromatic transition of *Dendrodoris temarana* from juvenile individuals of approximately 1 cm in length (A) to subadults measuring 2.5 cm (B, C, D) and adults of 4 cm (E, F, G) in the locality of Cubelles.



SUPPLEMENTARY TABLE 1. Records of *Dendrodoris temarana* from different naturalistic websites.

Locality	Reporter	Webpage
Étang de Thau (SE France)	P. Estachy	https://m.facebook.com/photo/?fbid=3147322218689978&set=a.567212116701014
Étang de Thau (SE France)	M. Bosch	https://opistobranquis.org/_usr/observations/20046/dendrodoris1.jpg
Étang de Thau (SE France)	P. Girard	https://www.youtube.com/watch?v=TlPxufcU4_U&feature=youtu.be
Étang de Thau (SE France)	X. Salvador	https://opistobranquis.org/_usr/observations/19913/DSC_0125.jpg
La Línea de la Concepción (S Spain)	D. Pereira	https://m.facebook.com/photo?fbid=3422281134482420&set=pcb.3114711331952717
Mallorca Islands (E Spain)	L. Gallego	https://m.facebook.com/photo/ ?fbid=10216449829812126&set=gm.10158547288013154
Ebro Delta (NE Spain)	J. Camps	https://i1.wp.com/opistobranquis.info/wp-content/uploads/2020/04/Dendrodoris-sp-by-Judith-Camps-DSCN7067-Delta.jpg?w=900&ssl=1
Cala d´Oques (NE Spain)	M. González	https://opistobranquis.org/_usr/observations/2340/P7090014.JPG
Port de l´Estany (NE Spain)	X. Salvador	https://opistobranquis.org/_usr/observations/19705/DSC_2157.jpg