Changing feeding habits and ontogenetic dimorphism in juveniles and adults Aplysia punctata (Cuvier, 1803) (Mollusca, Gastropoda, Heterobranchia) in the Mediterranean Sea

DARIO CÓRDOBA GONZÁLEZ, ALBA ENGUIDANOS, ÁNGEL VALDÉS, MANUEL BALLESTEROS

doi: 10.12681/mms.29735

To cite this article:
CÓRDOBA GONZÁLEZ, D., ENGUIDANOS, A., VALDÉS, ÁNGEL, & BALLESTEROS, M. (2022). Changing feeding habits and ontogenetic dimorphism in juveniles and adults Aplysia punctata (Cuvier, 1803) (Mollusca, Gastropoda, Heterobranchia) in the Mediterranean Sea. Mediterranean Marine Science, 23(4). https://doi.org/10.12681/mms.29735
Changing feeding habits and ontogenetic dimorphism in juveniles and adults *Aplysia punctata* (Cuvier, 1803) (Mollusca, Gastropoda, Heterobranchia) in the Mediterranean Sea

Dario CÓRDOBA GONZÁLEZ, Alba ENGUIDANOS, Ángel VALDÉS and Manuel BALLESTEROS

1 Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Avda. Diagonal, 643, 08028 Barcelona, Spain
2 Department of Biological Sciences, California State Polytechnic University, 3801 West Temple Avenue, Pomona, California 91768, USA

Corresponding author: Dario CÓRDOBA GONZÁLEZ; dcg213@cwpanama.net

Contributing Editor: Fabio CROCETTA

Received: 25 February 2022; Accepted: 27 August 2022; Published online: 11 October 2022

Abstract

Specimens of *Aplysia punctata* inhabiting the Catalan coast (NE Spain, Western Mediterranean Sea) display two different color patterns, which have been thought to represent two distinct species. However, molecular analyses conducted by other authors and confirmed herein show that there are no genetic differences between individuals of the two color morphs. At the same time, these color morphs display distinct life history traits, including different size, mating behavior, and egg mass and larval coloration. In this paper, it is hypothesized that distinct diets are responsible of these differences. The results of this study confirm that small red specimens of *A. punctata* feed on red algae and primarily *Sphaerococcus coronopifolius* and *Plocamium cartilagineum*; pigments from these algae give small specimens of *A. punctata* a very similar morphological appearance to adults of the *Aplysia parvula* species complex. In contrast, adult specimens of *A. punctata* feed preferentially on green algae of the species *Ulva lactuca*. This change in feeding behavior is probably related to defensive strategies involving camouflage. In addition, the egg masses take on a pink to reddish color that is retained in the larval stage of *A. punctata* juveniles.

Keywords: *Aplysia punctata*; dietary preference; dimorphism; coloration; Catalanian coast.

Introduction

The adoption of molecular phylogenetic methods has resulted in significant classification and taxonomic changes among diverse groups of living organisms (Field et al., 1988), confirming the limitations of morphological data for phylogenetic reconstruction (Doyle, 1992; Knowlton, 2000). Some examples involve species previously considered to have wide geographic distributions (Andrews et al., 2014; Gaither et al., 2016) or organisms with reduced morphological diversification (Fukami et al., 2004). Among the others, Cooke et al. (2014), Kienberger et al. (2016), Lindsay and Valdés (2016) indicated that molecular analyses have been very useful to clarify the classification of sea slugs in cases where several species with similar morphology could not be discerned by traditional methods, and were formerly considered to be single widespread species. In line with that, molecular analyses have confirmed that most species with large geographical ranges across different ocean basins are in fact pseudo-cryptic or cryptic species complexes of closely related taxa, and several authors provide hypothesi-

ses on the mechanisms of evolution resulting in their current distribution (Ornelas-Gatdula et al., 2012; Carmona et al., 2014; Valdés et al., 2017; Uribe et al., 2018).

Species of the genus *Aplysia* Linnaeus, 1767, known as sea hares (Heterobranchia, Aplysiida), live in the intertidal or upper subtidal zones where they feed, mate, and lay their egg masses on different species of algae (Ribero et al., 1998). According to Bouchet and Gofas (2022), there are around 45 species of *Aplysia* worldwide and many other species considered *nomina dubia* or *taxa inquirenda*. Species of *Aplysia* live in temperate or tropical waters, and no species has been able to colonize the cold waters of the Arctic or the Antarctic oceans. Medina and Walsh (2000) carried out the first phylogenetic analysis of the Aplysiida based on mitochondrial DNA, while Medina et al. (2001) carried out a preliminary phylogeny on the genus *Aplysia* in which they include *Aplysia parvula* (Mörch, 1863) and *Aplysia punctata* (Cuvier, 1803) as distinct species.

The identification of species of *Aplysia* based on morphological traits is problematic as some species are highly variable in external coloration (Medina & Walsh, 2000).
An example is *A. punctata*, a very common species in the Mediterranean Sea that displays high levels of chromatic diversity. Ballesteros and Templado (1987) misidentified small specimens of *A. punctata* as *A. parvula*, because of their reduced size and reddish coloration similar to that of *A. parvula*. Ballesteros and Templado (1987) also carried out a morphological study of these small *Aplysia*, analyzing their radular structure and providing data on their biology and reproduction. Since the paper of Ballesteros and Templado (1987), small specimens of *A. punctata* with lengths of 5-20 mm were cited in the western Mediterranean under the name of *A. parvula* (see Martin et al., 1990; Sánchez Tocino, 2001; Domènech et al., 2002; Cervera et al., 2004; Ballesteros, 2007; Coll et al., 2010; Ballesteros et al., 2016; Gofas et al., 2017), although sometimes with reservations (Crocetta et al., 2015; Corsini-Foka et al., 2015; Zenetos et al., 2016). Only recently, Golestani et al. (2019) found that there is no evidence of the presence of *A. parvula* in the Mediterranean Sea and that all specimens showing the *A. parvula* phenotype are *A. punctata* or possible hybrids. Finally, Ballesteros et al. (2021) provided additional information on these small red specimens of *A. punctata* with numerous photographs illustrating details of their coloration and morphology, as well as how this species changes coloration at different stages of its life cycle.

However, the boundaries in external morphology between the two-color morphs still needs to be clarified, as well as the reasons why the feeding strategy of individuals changes as their size increases. In this paper we attempt to address these questions by examining changes in feeding behavior during different stages of the life cycle of *A. punctata*, as well as the reproductive period, mating behavior, egg mass coloration, and other traits based on natural populations and individuals collected for observation in the laboratory. Moreover, molecular analyses were conducted to verify the identity of the specimens examined and confirm or refute the results by Golestani et al. (2019).

**Materials and Methods**

**Morphological analysis**

In this paper the abbreviature APJ is used for the small red specimens previously cited as *A. parvula* and the abbreviature APA is used for larger animals typically identified as *A. punctata*. Live specimens of APJ and APA with their egg masses were photographed live in the field or in the laboratory. A binocular Olympus SZ-PT dissecting microscope was used to study their internal anatomy. A dorsal incision was made on the anterior portion of the animal to dissect the distal anterior part of the digestive tract, which includes the buccal bulb, radula, and jaws. The buccal mass was immersed in a potassium hydroxide (KOH) solution (10%) for three hours to dissolve the organic tissues and then rinsed with distilled water. The radula was mounted on a metal stub with bio adhesive tabs, coated with a carbon layer, and examined under a FEI Quanta 200 scanning electron microscope (SEM).

**DNA extraction and amplification**

For the molecular analyses, 13 specimens of APJ and 13 of APA were collected by SCUBA diving or snorkeling in different locations on the Catalonian coast (NE Spain). Specimens were collected live in the field, immediately transferred to 99% ethanol and stored at -20°C for the subsequent DNA extraction. To complete the dataset, we retrieved all the available sequences of the *A. parvula* and *A. punctata* from Genbank, listed in Table 1. Four specimens of *Bursatella leachi* from Fòrum (Barcelona, Spain) and Ebro Delta (Tarragona, Spain) were used to root the tree.

Total genomic DNA was extracted from the foot of each specimen using the REDExtract-N-Amp™ Tissue-PCR Kit Protocol from Sigma-Aldrich and following the manufacturer’s protocol. Partial fragments of two mitochondrial genes and one nuclear gene were sequenced: COI (cytochrome c oxidase subunit I), 16S (ribosomal RNA) and Histone H3. The primers used for amplification are listed in Table 2. The LCO1490/HCO2198 was the preferred combination, while Nancy was used as a replacement for HCO2198 (Table 2).

The polymerase chain reaction (PCR) was performed using 5 μl 5× MyTaq Reaction Buffer (BIOLINE), 0.4 μl primers forward and reverse (10 μM), 0.2 μl MyTaq Red DNA Polymerase (BIOLINE), 4 μl of diluted DNA and ultrapure distilled water up to a total reaction volume of 20 μl. COI was amplified as a follows: 5 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature range of 42°C for 35 s, 72°C for 45 s and final extension at 72°C for 5 min; 16S and Histone H3 were amplified as a follows: 2 min at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 35 s and extension at 68°C for 1 min and final extension at 68°C for 7 min.

Fragments were visualized by agarose gel electrophoresis at a concentration of 1% (w/v) in 1× TBE buffer (0.89 M Tris, 0.89 M boric acid, 0.02 M EDTA) (Panreac AppliChem, Barcelona, Spain) at 110 V for 45 min. PCR products were sequenced in both directions using one of the respective amplification primers at Macrogen services (www.macrogen.com). The chromatograms were assembled and edited in Geneious Prime v2021.1.1. (Drummond et al., 2010).

**Phylogenetic analysis**

Different alignments were built for downstream phylogenetic analyses containing all the available *Aplysia fasciata* Poiret, 1789, *Aplysia nigrocincta* von Martens, 1880, and *A. punctata* sequences and four *Bursatella leachi* Blainville, 1817 sequences, including: the individual COI, 16S, and Histone H3 datasets; the concatenated mitochondrial alignment with COI and 16S sequences; and the concatenated alignment with mitochondrial (COI + 16S) and nuclear (Histone H3) sequences.

Maximum likelihood (ML) and Bayesian inference analysis (BI) phylogenetic analyses were conducted.
Table 1. Information on all the specimens used in this study for molecular phylogeny, including the specie, location, voucher (collection code) and the COI GenBank accession number.

| Specie                  | Location                                | Voucher | COI          | 16S          | H3          | Reference                  |
|-------------------------|-----------------------------------------|---------|--------------|--------------|-------------|----------------------------|
| B. leachii              | Fòrum, Barcelona, Spain                 | B1      | OK066363     | --------------| --------------| present study             |
| B. leachii              | Fòrum, Barcelona, Spain                 | B2      | OK066356     | --------------| --------------| present study             |
| B. leachii              | Ebro Delta, Tarragona, Spain            | B6      | OK066355     | --------------| --------------| present study             |
| B. leachii              | Ebro Delta, Tarragona, Spain            | B7      | OK066359     | --------------| --------------| present study             |
| APA                     | Roses (Costa Brava), Spain              | Ap1     | OK066334     | OK066287     | OK073542     | present study             |
| APA                     | Roses (Costa Brava), Spain              | Ap2     | OK066333     | OK066286     | OK073541     | present study             |
| APA                     | Fòrum, Barcelona, Spain                 | Ap3     | --------------| OK066285     | OK073540     | present study             |
| APA                     | Fòrum, Barcelona, Spain                 | Ap4     | OK066284     | --------------| --------------| present study             |
| APA                     | Fòrum, Barcelona, Spain                 | Ap5     | --------------| OK073539     | --------------| present study             |
| APA                     | Cala Fosca (Costa Brava), Spain         | Ap6     | OK066332     | OK066283     | OK073538     | present study             |
| APA                     | Cala Fosca (Costa Brava), Spain         | Ap7     | OK066331     | OK066282     | OK073537     | present study             |
| APA                     | Es Caials (Costa Brava), Spain          | Ap8     | --------------| OK066281     | OK073536     | present study             |
| APA                     | Cala Sant Antoni (Costa Brava), Spain   | Ap9     | OK066330     | OK066280     | OK073535     | present study             |
| APA                     | Fòrum, Barcelona, Spain                 | Ap10    | OK066279     | OK073534     | --------------| present study             |
| APA                     | Fòrum, Barcelona, Spain                 | Ap11    | OK066278     | OK073533     | --------------| present study             |
| APA                     | Rosas (Costa Brava), Spain              | AR      | OK066337     | OK050558     | OK073519     | present study             |
| APA                     | Rosas (Costa Brava), Spain              | AR1     | --------------| OK050559     | OK073520     | present study             |
| APJ                     | Bau Cap Falco (Costa Brava), Spain      | A1      | OK066349     | OK050571     | --------------| present study             |
| APJ                     | Bau Cap Falco (Costa Brava), Spain      | A2      | OK066348     | OK050570     | OK073532     | present study             |
| APJ                     | Cala Fosca (Costa Brava), Spain         | A3      | OK066336     | OK050569     | OK073531     | present study             |
| APJ                     | Cala Aiguablava-Fornells (Costa Brava), Spain | A4 | OK066347 | OK050568 | OK073530 | present study             |
| APJ                     | Cala Aiguablava-Fornells (Costa Brava), Spain | A5 | OK066340 | OK050567 | OK073529 | present study             |
| APJ                     | Mataró, Spain                           | A6      | OK066339     | OK050566     | OK073528     | present study             |
| APJ                     | Mataró, Spain                           | A7      | OK066346     | --------------| OK073527     | present study             |
| APJ                     | Mataró, Spain                           | A8      | OK066345     | OK050565     | OK073526     | present study             |
| APJ                     | Mataró, Spain                           | A9      | OK066344     | OK050564     | OK073525     | present study             |
| APJ                     | Port Illigat (Costa Brava), Spain       | A10     | OK066338     | OK050563     | OK073524     | present study             |
| APJ                     | Fòrum, Barcelona, Spain                 | A11     | OK066343     | OK050562     | OK073523     | present study             |
| APJ                     | Fòrum, Barcelona, Spain                 | A12     | OK066335     | OK050561     | OK073522     | present study             |
| APJ                     | Fòrum, Barcelona, Spain                 | A13     | OK066342     | OK050560     | OK073521     | present study             |
| A. fasciata             | Israel                                  | --------| AF343428     | AF192298     | --------------| Medina et al., 2005      |
| A. fasciata             | Sao Sebastiao, Sao Paulo, Brazil (1)     | MZSP 103234 | KM272290 | KM272281 | --------------| de Oliveira et al., 2014 |

Continued
**Table 1 continued**

| Specie       | Location                      | Voucher          | COI       | 16S       | H3         | Reference              |
|--------------|-------------------------------|------------------|-----------|-----------|------------|------------------------|
| *A. fasciata*| Sao Sebastian, Sao Paulo, Brazil (2) | MZSP 103233  | KM272291 | KM272282 |------------| de Oliveira et al., 2014 |
| *A. fasciata*| Sao Sebastian, Sao Paulo, Brazil (3) | MZSP 103250  | KM272292 | KM272283 |------------| de Oliveira et al., 2014 |
| *A. fasciata*| Sao Sebastian, Sao Paulo, Brazil (4) | MZSP 103217  | KM272295 |------------|------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (1) | MZSP 109903  | KM272284 |------------|------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (2) | MZSP 113776  | KM272293 | KM272285 |------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (3) | MZSP 109975  | KM272286 |------------|------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (4) | MZSP 104038  | KM272287 |------------|------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (5) | MZSP 113777  | KM272288 |------------|------------| de Oliveira et al., 2014 |
| *A. fasciata*| Ponta da Praia, Santos, Sao Paulo, Brazil (6) | MZSP 113777  | KM272289 |------------|------------| de Oliveira et al., 2014 |
| *A. nigrocincta*| Florida, USA | -------------- | AF343426  | AF192296 |------------| Medina et al., 2005 |
| *A. nigrocincta*| Madang, Papua New Guinea | CASIZ 191092  | MK422792 |------------|------------| Golestani et al., 2019 |
| *A. punctata*| Espiritu Santo, Vanuatu | CASIZ 176792  | MK422893 | MK422693 |------------| Golestani et al., 2019 |
| *A. punctata*| North Sea (1) | -------------- | KR084671 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (2) | -------------- | KR084707 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (3) | -------------- | KR084714 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (4) | -------------- | KR084740 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (5) | -------------- | KR084809 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (6) | -------------- | KR084865 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (7) | -------------- | KR084890 |------------|------------| Barco et al., 2016 |
| *A. punctata*| North Sea (8) | -------------- | KR084908 |------------|------------| Barco et al., 2016 |
| *A. punctata*| Norwegian archipelago | -------------- | KT952472 |------------|------------| Ware et al., 2015 |
| *A. punctata*| Azores, Portugal (1) | CPIC 01363    | MK422894 | MK422795 | MK422695 | Golestani et al., 2019 |
| *A. punctata*| Azores, Portugal (2) | CPIC 01364    | MK422895 | MK422796 | MK422696 | Golestani et al., 2019 |
| *A. punctata*| Azores, Portugal (3) | CPIC 01366    | MK422896 | MK422798 | MK422698 | Golestani et al., 2019 |
| *A. punctata*| Azores, Portugal (4) | CPIC 01365    | MK422797 | MK422697 |------------| Golestani et al., 2019 |
| *A. punctata*| Azores, Portugal (5) | CPIC 01356    | MK422794 | MK422694 |------------| Golestani et al., 2019 |
| *A. punctata*| Cadiz, Spain | CPIC 01359    | MK422897 |------------|------------| Golestani et al., 2019 |
| *A. punctata*| Santander, Spain | CPIC 01424    | MK422898 | MK422799 | MK422700 | Golestani et al., 2019 |
| *A. punctata*| Pontevedra, NW Spain | -------------- | AY345019 |------------|------------| Grande et al., 2004 |
| *A. punctata*| Brittany, France (1) | ZSM Mol 20180005 | MK422899 | MK422800 | MK422701 | Golestani et al., 2019 |
| *A. punctata*| Brittany, France (2) | ZSM Mol 20180005 | MK422900 | MK422801 | MK422702 | Golestani et al., 2019 |
|              |                 |                 |          |          |            | **Continued** |

830 Medit. Mar. Sci., 23/4 2022, 827-849
| Specie       | Location                  | Voucher      | COI          | 16S          | H3          | Reference                        |
|--------------|----------------------------|--------------|--------------|--------------|-------------|----------------------------------|
| A. punctata  | Brittany, France (3)      | ZSM Mol 20180006 | MK422901 | MK422802 | MK422703  | Golestani et al., 2019           |
| A. punctata  | Banyuls sur Mer, France (1)| CPIC 01360   | MK422902 | MK422803 | MK422704  | Golestani et al., 2019           |
| A. punctata  | Banyuls sur Mer, France (2)| CPIC 01360   | MK422903 | MK422804 | MK422705  | Golestani et al., 2019           |
| A. punctata  | Banyuls sur Mer, France (3)| CPIC 01362   | MK422904 | MK422805 | MK422706  | Golestani et al., 2019           |
| A. punctata  | Giannutri Island, Italy (1)|           |            |            | MK422806  | Golestani et al., 2019           |
| A. punctata  | Giannutri Island, Italy (2)|           |            |            | MK422807  | Golestani et al., 2019           |
| A. punctata  | Giannutri Island, Italy (3)|           |            |            | MK422808  | Golestani et al., 2019           |
| A. punctata  | Giannutri Island, Italy (4)| CPIC 01422   | MK422908 | MK422809 | MK422709  | Golestani et al., 2019           |
| A. punctata  | Livorno, Italy (1)         |              |            |            | MK422810  | Golestani et al., 2019           |
| A. punctata  | Livorno, Italy (2)         |              |            |            | MK422811  | Golestani et al., 2019           |
| A. punctata  | Capraia Island, Italy (1)  |              |            |            | MK422813  | Golestani et al., 2019           |
| A. punctata  | Capraia Island, Italy (2)  |              |            |            | MK422812  | Golestani et al., 2019           |
| A. punctata  | Capraia Is., Italy (3)     | CPIC 01423   | MK422814 | MK422713 |           | Golestani et al., 2019           |
| A. punctata  | Gallipoli, Italy (1)       | CPIC 01353   |            | MK422815  | MK422714  | Golestani et al., 2019           |
| A. punctata  | Gallipoli, Italy (2)       | CPIC 01358   |            | MK422816  | MK422716  | Golestani et al., 2019           |
| A. punctata  | Gallipoli, Italy (3)       | CPIC 01357   |            | MK422817  | MK422715  | Golestani et al., 2019           |
| A. punctata  | Levanto, Italy             | CPIC 01421   | MK422912 | MK422817 | MK422717  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (1)         |              |            | MK422913  | MK422818  | MK422718  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (2)         |              |            | MK422914  | MK422819  | MK422719  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (3)         |              |            | MK422915  | MK422820  | MK422720  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (4)         |              |            | MK422916  | MK422821  | MK422721  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (5)         |              |            | MK422917  | MK422822  | MK422722  | Golestani et al., 2019           |
| A. punctata  | Rhodes, Greece (6)         |              |            | MK422918  | MK422823  | MK422723  | Golestani et al., 2019           |
| A. punctata  | Heraklion, Crete, Greece   | CPIC 01388   | MK422919  | MK422824  | MK422724  | Golestani et al., 2019           |
| A. punctata  | Cape Kamenjak, Croatia (1) | ZSM Mol 20090003-1 | MK422920 | MK422825 | MK422725  | Golestani et al., 2019           |
| A. punctata  | Cape Kamenjak, Croatia (2) | ZSM Mol 20090003-2 | MK422921 | MK422826 | MK422726  | Golestani et al., 2019           |
| A. punctata  | Cape Kamenjak, Croatia (3) | ZSM Mol 20090003-3 | MK422922 | MK422827 | MK422727  | Golestani et al., 2019           |
| A. punctata  | Cape Kamenjak, Croatia (4) |              |            | MK422923  | MK422828  | MK422728  | Golestani et al., 2019           |
| A. punctata  | Umago, Croatia             | CPIC 01374   | MK422913  | MK422829  | MK422729  | Golestani et al., 2019           |
| A. punctata  | Helgoland, North Sea       |              |            | AF249253  |            | Wollscheid-Lengeling et al., 2001 |
| A. punctata  | Southwest Turkey (1)       |              |            | MF784857  |            | Yokes, 2018                      |
| A. punctata  | Southwest Turkey (2)       |              |            | MF784858  |            | Yokes, 2018                      |

Continued
using the best partition scheme and evolutionary model for each partition selected with Partition Finder v2.1.1. (Lanfear et al., 2017). ML analyses were performed with RAxML 8.0 (Stamatakis, 2014) with 10000 replicates, random initial trees, and estimated parameters evolutionary models. Branches with bootstrap values above 70% were considered supported (Hillis & Bull, 1993). The first 10% of the generations were discarded as a burn-in for the analyses. Posterior probability node values (PP) higher than 0.9 were considered supported (Huelsenbeck & Rannala, 2004). The resulting trees were visualized with iToL (https://itol.embl.de/itol.cgi).

Table 1 continued

| Specie             | Location            | Voucher       | COI            | 16S             | H3              | Reference     |
|-------------------|---------------------|---------------|----------------|-----------------|-----------------|---------------|
| A. punctata       | Southwest Turkey (3)| --------------| MF784859       | ---------------| MK184487        | Yokes, 2018   |
| A. punctata       | Southwest Turkey (4)| MF784860      | ---------------| MK184488        | Yokes, 2018     |
| A. punctata       | Southwest Turkey (5)| MF784861      | ---------------| MK184490        | Yokes, 2018     |
| A. punctata       | Southwest Turkey (6)| MF784862      | ---------------| MK184491        | Yokes, 2018     |
| A. punctata       | Southwest Turkey (7)| MF784863      | ---------------| MK184492        | Yokes, 2018     |
| A. punctata       | Southwest Turkey (8)| MF784864      | ---------------| MK184493        | Yokes, 2018     |
| A. punctata       | Southwest Turkey (9)| --------------| ---------------| MK184489        | Yokes, 2018     |

Table 2. Primers used for each gene.

| Gene | Primers Name | Direction | Secuencia | Reference |
|------|--------------|-----------|-----------|-----------|
| COI  | LCO1490      | Forward   | 5′-GGTCAACAAATCATAAAGATATTGG-3′ | Folmer et al. 1994 |
| COI  | HCO2198      | Reverse   | 5′-TAAACTTCAGGGTGACCCAAAAATACTA-3′ | Folmer et al. 1994 |
| COI  | Nancy        | Reverse   | 5′-CCCGGTAATAATCTATACTTACAT-3′ | Simon et al. 1994 |
| 16S  | 16Sar        | Forward   | 5′-CGCCTGTATTCAAAAACAT-3′ | Palumbi et al. 1991 |
| 16S  | 16Sbr        | Reverse   | 5′-CCGGTYGAATCTACATCAYGT-3′ | Palumbi et al. 1991 |
| H3   | H3F          | Forward   | 5′-ATGGCTCGTACCAAAGGACVGC-3′ | Bernhard 1999     |
| H3   | H3R          | Reverse   | 5′-CTTCTGCGAGTTGCTAT-3′ | Bernhard 1999     |

**Biological data**

Most of the observations of APJ and APA have been carried out in different locations on the Costa Brava (NE Spain) (Fig. 1). Data on the biology of APJ and APA in the Catalonian coast is based on long-term field observations by one of the authors (MB), compiled for more than 40 years. Moreover, records of specimens from other sources, mainly from members of the GROC (Grup de Recerca dels Opistobranquis de Catalunya) as well as photographs published on the internet have been examined. All data are provided in Table 3. Data collected include the color pattern of the specimen, date, locality. When possible, the number of specimens, the substrate, and any noteworthy behavior such as copulation and the presence of egg masses, were recorded both in the field observations or photographs. In total, all the observational data correspond to approximately 150 hours of scuba diving by different divers. Due to the small size and camouflage of APJ specimens, masses of frondose red algae such as *Sphaerococcus coronopifolius* Stackhouse and *Plocamium cartilagineum* (Linnaeus) P.S. Dixon were collected in different locations and the specimens were separated from the algae in the laboratory, by placing them in small-capacity aquaria. Some specimens of APJ and APA were induced to ink by touching them, others were collected with their respective algae that serve as food and habitat to observe their behavior in the laboratory.
Fig. 1: Specimens capture locations of APJ and APA. In red the zone of the Costa Brava that is between 41.84194°N, 3.10583°W and 42.43638°N, 317611°W.

Table 3. Observations of APJ (as A. parvula) and APA on the Catalonian coast. Notes: the observations not reported by M. Ballesteros, M. Pontes and E. Madrenas correspond to members of the GROC (Grup de Recerca dels opistobranquis de Catalunya, https://opistobranquis.org/es/home) and are documented with photographs. CB= localities on the Costa Brava. * means observation not quantified in number of individuals. Blank substrate cells = no data.

| Species/Size | Observer       | date        | locality                        | other data | substrate                  |
|--------------|----------------|-------------|---------------------------------|------------|----------------------------|
| APJ          | M. Ballesteros | 27/02/1985  | Punta de Santa Anna (Blanes, CB) | *          | on Codium and Halopteris   |
| APJ          | M. Pontes      | 01/03/1985  | Punta de Santa Anna (Blanes, CB) | *          |                            |
| APJ          | M. Pontes      | 27/05/1985  | Pedra de Deu (Medes Is., CB)    | *          |                            |
| APJ          | M. Pontes      | 27/05/1985  | Punta de Santa Anna (Blanes, CB) | *          |                            |
| APJ          | M. Ballesteros | 27/05/1985  | Meda Gran (Illes Medes, CB)     | *          | on hidrarians and red algae |
| APJ          | M. Ballesteros | 07/06/1985  | Mar Menuda (Tossa de Mar, CB)   | *          |                            |
| APJ          | M. Pontes      | 09/03/1986  | Mar Menuda (Tossa de Mar, CB)   | *          |                            |
| APJ          | M. Ballesteros | 12/05/1999  | Cadaqués, CB                    | *          | copulate                   |
| APJ          | M. Ballesteros | 22/05/2000  | Roses, CB                       | *          |                            |
| APJ          | M. Pontes      | 01/02/2002  | Illa Mateua (L. Escala, CB)     | *          |                            |
| APJ          | M. Ballesteros | 21/03/2002  | Punta de Santa Anna (Blanes, CB)| *          |                            |
| APJ          | M. Ballesteros | 15/05/2002  | Es Caials (Cadaqués, CB)        | 1          | between red algae          |
| APJ          | M. Ballesteros | 17/05/2002  | L’Almadrava (Roses, CB)         | 2          | between algae              |

Continued
### Table 3 continued

| Species/Size | Observer | date       | locality                           | other data | substrate  |
|--------------|----------|------------|------------------------------------|------------|------------|
|              | APJ      | 21/05/2003 | Es Caials (Cadaqués, CB)           | 1          |            |
|              | APJ      | 23/05/2003 | Punta de la Creu (Roses, CB)       | 1          |            |
|              | APJ      | 14/05/2004 | Cala dels Gats (Palamós, CB)       | *          |            |
|              | APJ      | 24/05/2004 | Punta de Santa Anna (Blanes, CB)   | *          |            |
|              | APJ      | 16/05/2005 | L’Almadrava (Roses, CB)            | *          |            |
|              | APJ      | 26/01/2008 | Es Caials, CB                      | *          |            |
|              | APJ      | 02/02/2008 | Es Caials, CB                      | *          |            |
|              | APJ      | 02/03/2008 | Illa Mateua (L’Escala, CB)         | *          |            |
|              | APJ      | 03/05/2008 | Es Caials, CB                      | *          |            |
|              | APJ      | 19/05/2008 | Bau Cap Falcó (Roses, CB)          | *          |            |
|              | APJ      | 18/05/2009 | El Bisbe de Norfeu, CB             | 1          |            |
|              | APJ      | 19/05/2010 | Punta Falcó, CB                    | 7          | between algae |
|              | APJ      | 20/02/2010 | Es Caials, CB                      | *          |            |
|              | APJ      | 23/02/2010 | Cala Sant Francesc (Blanes, CB)    | *          | on *Halopteris* |
|              | APJ      | 23/02/2010 | Cala Sant Francesc (Blanes, CB)    | *          | on *Sphaerococcus* |
|              | APJ      | 06/03/2010 | Es Caials, CB                      | *          |            |
|              | APJ      | 06/03/2010 | Mar Menuda (Tossa de Mar, CB)      | *          |            |
|              | APJ      | 17/04/2010 | Cala Aiguafreda, CB                | *          |            |
|              | APJ      | 03/07/2010 | Ferranelles (Illes Medes, CB)      | *          |            |
|              | APJ      | 22/01/2011 | Es Caials, CB                      | *          |            |
|              | APJ      | 16/04/2011 | La Caleta (Palamós, CB)            | *          |            |
|              | APJ      | 23/05/2011 | Mar Menuda (Tossa de Mar, )        | *          | on *Halopteris* |
|              | APJ      | 28/05/2011 | Es Caials (Cadaqués, CB)           | *          |            |
|              | APJ      | 21/04/2012 | Pota del Llop (Medes Is., CB)      | *          |            |
|              | APJ      | 21/05/2012 | El Bisbe de Norfeu, CB             | 11         |            |
|              | APJ      | 04/06/2012 | Es Caials (Cadaqués, CB)           | *          |            |
|              | APJ      | 05/06/2012 | Cap Norfeu (Roses, CB)             | *          |            |
|              | APJ      | 30/06/2012 | El Port de la Selva, CB            | *          |            |
|              | APJ      | 10/11/2012 | Salpatxot (Medes Is., CB)          | *          |            |
|              | APJ      | 18/01/2013 | Punta del Romani (L’Escala, CB)    | *          |            |
|              | APJ      | 17/04/2013 | Mataró                             | *          |            |
|              | APJ      | 07/05/2013 | Mar Menuda (Tossa de Mar, CB)      | 1          |            |

*Continued*
| Species/Size | Observer | date       | locality                          | other data | substrate       |
|-------------|----------|------------|-----------------------------------|------------|-----------------|
| APJ E. Madrenas | 19/05/2013 | Punta del Romani (L’Escala, CB) | *          |            |
| APJ M. Ballesteros | 23/05/2013 | Els Caials (Cadaqués, CB) | *          | on Sphaerococcus |
| APJ M. Ballesteros | 10/06/2013 | Punta Falconera (Roses, CB) | 1          |            |
| APJ M. Ballesteros | 11/06/2013 | El Gat de Norfeu (Roses, CB) | 1          |            |
| APJ E. Madrenas | 25/06/2013 | Es collera Port (Tarragona) | 3          | on Codium    |
| APJ E. Madrenas | 05/07/2013 | Cala dels Gats (Palamós, CB) | *          |            |
| APJ X. Salvador | 10/12/2013 | Palamós, CB | *          |            |
| APJ X. Salvador | 14/12/2013 | Cala Bramant, CB | *          |            |
| APJ X. Salvador | 04/01/2014 | Llançà, CB | *          |            |
| APJ M. Pontes | 22/02/2014 | Cala Aiguafrèda, CB | *          |            |
| APJ M. Pontes | 08/03/2014 | Cala Aiguafrèda, CB | *          |            |
| APJ M. Pontes | 18/04/2014 | Cala Margarida, CB | *          |            |
| APJ M. Pontes | 26/04/2014 | Cala Sa Tuna (Begur, CB) | *          |            |
| APJ G. Moreira | 14/05/2014 | La Caleta (Palamós, CB) | *          | on Sphaerococcus |
| APJ M. Ballesteros | 26/05/2014 | Punta de la Creu (Roses, CB) | 2          |            |
| APJ M. Ballesteros | 10/06/2014 | Bau de Punta Falconera (Roses, CB) | 15         | on Sphaerococcus |
| APJ M. Ballesteros | 11/06/2014 | Punta de la Creu (Roses, CB) | 1          |            |
| APJ X. Lindo | 06/09/2014 | Cala Margarida (Palamós, CB) | *          | on Flabellia |
| APJ M. Pontes | 24/01/2015 | Es Caials (Cadaqués, CB) | *          |            |
| APJ M. Pontes | 28/02/2015 | Es Caials (Cadaqués, CB) | *          |            |
| APJ M. Pontes | 14/03/2015 | Es Caials (Cadaqués, CB) | *          |            |
| APJ M. Pontes | 16/05/2015 | Es Caials (Cadaqués, CB) | *          |            |
| APJ M. Ballesteros | 25/05/2015 | Bau de Punta Falconera (Roses, CB) | 5          |            |
| APJ M. Ballesteros | 26/05/2015 | Es Caials (Cadaqués, CB) | 3          |            |
| APJ M. Ballesteros | 08/06/2015 | La Trona (Roses, CB) | 3          |            |
| APJ M. Ballesteros | 09/06/2015 | Punta Falconera (Roses, CB) | 7          |            |
| APJ X. Salvador | 14/07/2015 | Les Soffereres (Sant Feliu de Guixols, CB) | *          |            |
| APJ G. Mas | 12/09/2015 | Sa Tuna, CB | *          |            |
| APJ F. Asensio | 29/11/2015 | Es Caials (Cadaqués, CB) | *          |            |
| APJ E. Madrenas | 05/12/2015 | Punta del Falaguer (Medes Is., CB) | *          |            |
| APJ E. Madrenas | 16/04/2016 | Salputxot (Medes Is., CB) | *          |            |
| APJ M. Ballesteros | 23/05/2016 | Cap Falcó (Roses, CB) | 1          |            |
| APJ M. Pontes | 14/01/2017 | Es Caials (Cadaqués, CB) | *          |            |

Continued
Table 3 continued

| Species/Size | Observer   | date     | locality                     | other data                  | substrate           |
|--------------|------------|----------|------------------------------|---------------------------|---------------------|
| APJ          | X. Salvador | 02/03/17 | La Caleta (Palamós, CB)      | *                         |                     |
| APJ          | M. Casanovas | 06/03/17 | Tossa de Mar, CB             | *                         | on green algae      |
| APJ          | X. Salvador | 15/04/17 | Llafrenc, CB                 | *                         | on red algae        |
| APJ          | X. Salvador | 18/04/17 | Cala Aiguaxelida, CB         | *                         | on red algae        |
| APJ          | X. Salvador | 08/05/17 | Cala Ventosa (Sant Feliu de Guixols, CB) | * | on red algae |
| APJ          | S. Fuertes  | 20/05/17 | El Guix (Medes Is., CB)      | *                         |                     |
| APJ          | X. Salvador | 26/12/17 | Cala Montgó (L´Escala, CB)   | *                         | on Sphaerococcus    |
| APJ          | X. Salvador | 31/01/18 | Cala Aiguafreda, CB          | *                         |                     |
| APJ          | X. Salvador | 08/03/18 | Cala Sa Tuna, CB             | *                         | on Ulva             |
| APJ          | X. Salvador | 24/04/18 | Punta del Romani (L´Escala, CB) | * | on red algae |
| APJ          | X. Salvador | 27/04/18 | El Fòrum (Barcelona)         | *                         | on red algae        |
| APJ          | G. Biscop   | 16/05/18 | Tascó Gros (Medes Is., CB)   | *                         | on green algae      |
| APJ          | X. Salvador | 25/05/18 | Cala Aiguafreda, CB          | *                         | on red algae        |
| APJ          | M. Ballesteros | 04/06/18 | Cap Falcó (Roses, CB)       | 2                         | on Sphaerococcus    |
| APJ          | M. Ballesteros | 05/06/18 | La Trona (Roses, CB)        | 5                         | on Sphaerococcus    |
| APJ          | A. Mares    | 05/07/18 | Cala El Bofill (Cadaqués, CB) | * |                     |
| APJ          | J. Fernández | 08/08/18 | Platja del Pas (El Port de la Selva, CB) | * |                     |
| APJ          | X. Lindo    | 09/02/19 | Cala Sa Tuna, CB             | *                         |                     |
| APJ          | X. Salvador | 13/03/19 | Tamariu, CB                  | *                         |                     |
| APJ          | M. Ballesteros | 21/05/19 | El Bisbe (Roses, CB)        | 5                         | on Sphaerococcus    |
| APJ          | M. Ballesteros | 27/05/19 | Bau Cap Falcó (Roses, CB)   | 4                         | on Sphaerococcus    |
| APJ          | M. Ballesteros | 28/05/19 | Cala Sant Antoni (Cadaqués, CB) | 4 | on Sphaerococcus    |
| APJ          | M. Ballesteros | 03/06/19 | Bau Cap Falcó (Roses, CB)   | 3                         |                     |
| APJ          | X. Salvador | 07/05/20 | Punta d’en Bosch (Sant Feliu de Guixols, CB) | * |                     |
| APJ          | X. Salvador | 30/01/21 | Coves Cala Maset (Sant Feliu de Guixols, CB) | * |                     |
| APJ          | M. Ballesteros | 25/05/21 | Bau Cap Falcó (Roses, CB)   | 13                        |                     |
| APA          | M. Ballesteros | 26/05/77 | Cubelles                    | 61 + egg masses + copulate | under stones, on Dictyota |
| APA          | M. Ballesteros | 09/11/78 | Mar Menuda (Tossa de Mar, CB) | * |                     |
| APA          | M. Ballesteros | 14/03/79 | Punta de Santa Anna (Blanes, CB) | * |                     |
| APA          | M. Ballesteros | 25/05/79 | Cubelles                    | *                         | under stones        |
| APA          | M. Ballesteros | 14/06/79 | Cubelles                    | *                         | under stones        |
| APA          | M. Ballesteros | 25/11/79 | Cala Sant Francesc (Blanes, CB) | * |                     |

Continued
| Species/Size | Observer                | date       | locality                  | other data | substrate      |
|-------------|-------------------------|------------|----------------------------|------------|----------------|
| APA         | M. Ballesteros          | 10/04/1982 | Cubelles                  | *          | under stones   |
| APA         | M. Ballesteros          | 11/04/1982 | Cubelles                  | *          | under stones   |
| APA         | M. Ballesteros          | 22/05/1985 | Cubelles                  | *          | under stones   |
| APA         | M. Ballesteros          | 07/05/1986 | Cubelles                  | *          | under stones   |
| APA         | M. Ballesteros          | 01/05/1993 | Roses, CB                 |            |                |
| APA         | M. Pontes               | 01/08/2000 | Torre Valentina (Calonge, CB) | *          |                |
| APA         | M. Ballesteros          | 06/05/2002 | Cala Fosca (Palamós, CB)  | *          |                |
| APA         | M. Ballesteros          | 27/08/2003 | Punta de Santa Anna (Blanes, CB) | *          |                |
| APA         | M. Ballesteros          | 28/08/2003 | Punta de Santa Anna (Blanes, CB) | *          |                |
| APA         | M. Ballesteros          | 18/05/2005 | Es Caials (Cadaqués, CB)  | 1          |                |
| APA         | M. Pontes               | 08/04/2006 | Es Caials (Cadaqués, CB)  | *          |                |
| APA         | E. Madrenas             | 20/02/2010 | Sa Tuna (Begur, CB)       | *          |                |
| APA         | E. Madrenas             | 27/03/2010 | La Caleta (Palamós, CB)   | *          |                |
| APA         | A. López-Arenas         | 29/03/2010 | La Caleta (Palamós, CB)   | *          |                |
| APA         | GROC                    | 03/07/2010 | Ferranelles (Illes Medes, CB) | *          |                |
| APA         | A. López-Arenas         | 25/02/2012 | Cala Aiguafreda, CB       | *          |                |
| APA         | M. Ballesteros          | 22/05/2012 | Es Caials (Cadaqués, CB)  | 1 juv.     | under stones   |
| APA         | M. Ballesteros          | 01/06/2012 | Cala Fosca (Palamós, CB)  | *          |                |
| APA         | M. Ballesteros          | 05/06/2012 | Cap Norfeu (Roses, CB)    | *          |                |
| APA         | E. Madrenas             | 25/11/2012 | Punta del Romani (L’Escala, CB) | *          |                |
| APA         | E. Madrenas             | 25/11/2012 | Punta del Romani (L’Escala, CB) | *          |                |
| APA         | E. Madrenas             | 26/03/2013 | La Caleta (Palamós, CB)   | *          |                |
| APA         | E. Madrenas             | 31/03/2013 | Aiguafreda (Begur, CB)    | *          |                |
| APA         | M. Ballesteros          | 28/05/2013 | Cala Fosca (Palamós, CB)  | 3          | under stones   |
| APA         | M. Ballesteros          | 29/05/2013 | Cala Fosca (Palamós, CB)  | 3          | under stones   |
| APA         | E. Madrenas             | 01/06/2013 | Cala Margarida (Palamós, CB) | *          |                |
| APA         | E. Madrenas             | 25/06/2013 | Barcelona breakwater      | *          |                |
| APA         | M. Pontes               | 29/03/2014 | Cala Sa Tuna (Begur, CB)  | *          |                |
| APA         | X. Salvador             | 11/04/2014 | La Llosa (Palamós, CB)    | *          |                |
| APA         | G. Mas                  | 02/08/2014 | Cala Margarida, CB        | *          |                |
| APA         | X. Salvador             | 10/04/2015 | Cova d’Ariadna (Sa Riera, CB) | *          | on algae       |
| APA         | E. Madrenas             | 18/04/2015 | Tascons Petits (Medes Is., CB) | *          |                |
| APA         | M. Ballesteros          | 26/05/2015 | Es Caials (Cadaqués, CB)  | 3          | under stones   |

Continued
Table 3 continued

| Species/Size | Observer        | date       | locality                  | other data       | substrate     |
|--------------|-----------------|------------|---------------------------|------------------|---------------|
| APA M. Ballesteros | 27/05/2015      | Cala Fosca (Palamós, CB) | *               | under stones   |
| APA M. Ballesteros | 01/07/2015      | Cubelles   | 12                       | under stones     |
| APA M. Badia  | 28/07/2015      | Sant Pol   | *                         |                 |
| APA M. Codina | 01/05/2016      | Cala Santa Anna, CB | *               |                 |
| APA M. Ballesteros | 24/05/2016      | Es Caials (Cadaqués, CB) | 10+ copulate | under stones   |
| APA M. Ballesteros | 25/05/2016      | La Fosca (Palamós, CB) | 11+ eggs masses | under stones   |
| APA M. Ballesteros | 27/05/2016      | La Fosca (Palamós, CB) | 10             | under stones   |
| APA M. Ballesteros | 8/06/2016       | Es Caials (Cadaqués, CB) | 4+ copulate   | under stones   |
| APA X. Salvador | 18/04/2017      | Cala Aiguaxelida, CB | *              | on algae       |
| APA E. Madrenas | 18/11/2017      | Llosa del Falaguer (Medes Is., CB) | *           |                 |
| APA M. Bosch   | 29/12/2017      | La Gavina (Sant Feliu de Guixols, CB) | *          |                 |
| APA X. Salvador | 27/04/2018      | El Fòrum (Barcelona) | *              |                 |
| APA A. Mares   | 16/05/2018      | Cala El Bofill (Cadaqués, CB) | *          |                 |
| APA G. Alvarez | 16/05/2018      | El Forum (Barcelona) | *              |                 |
| APA M. Ballesteros | 17/05/2018      | El Forum (Barcelona) | >150+ copulate | under stones   |
| APA M. Ballesteros | 24/05/2018      | La Fosca (Palamós, CB) | 7              | under stones   |
| APA X. Salvador | 24/05/2018      | Punta del Romani (L’Escala, CB) | *         |                 |
| APA X. Salvador | 25/05/2018      | Cala Aiguafreda, CB | *              | on red algae   |
| APA M. Ballesteros | 04/06/2018      | Cap Falcó (Roses, CB) | 1              |                 |
| APA M. Ballesteros | 06/06/2018      | Es Caials (Cadaqués, CB) | 5              |                 |
| APA M. Ballesteros | 05/07/2018      | El Fòrum (Barcelona) | 7              | under stones   |
| APA M. Ballesteros | 12/07/2018      | El Fòrum (Barcelona) | *              |                 |
| APA X. Salvador | 09/03/2019      | Cala Sa Tuna, CB | *              |                 |
| APA A. Parera   | 02/04/2019      | El Fòrum (Barcelona) | *              |                 |
| APA M. Bosch    | 16/04/2019      | Cala Aiguablava, CB | *              | on algae       |
| APA A. Parera   | 13/05/2019      | El Fòrum (Barcelona) | *              | on red algae   |
| APA M. Ballesteros | 22/05/2019      | Es Caials (Cadaqués, CB) | 1            | under stones   |
| APA M. Ballesteros | 24/05/2019      | La Fosca (Palamós) | 13+ egg masses | under stones   |
| APA M. Ballesteros | 05/06/2019      | Es Caials (Cadaqués, CB) | 5            | under stones   |
| APA A. Parera   | 15/06/2019      | El Fòrum (Barcelona) | *              |                 |
| APA J. Vilanova | 06/07/2019      | Tamariu, CB | *              |                 |
| APA C. Escarré  | 13/07/2019      | El Fòrum (Barcelona) | *              |                 |

Continued
feeding and reproductive behavior, as well as the deposition of egg masses, were observed in the laboratory.

Results

Phylogenetic trees

The mitochondrial alignment and the concatenated COI, 16S and H3 alignment included, respectively, 1113 bases and 1441 bases with 103 sequences. All the trees designed showed a topology with a similar or very similar behavior, giving us Bayesian analysis as maximum likelihood where clades were recovered; well defined as that of *A. fasciata* from the western Atlantic with Israel (Bs = 87), as well as another well-defined clade from *A. nigrocincta* from the Indo-Pacific (Bs = 97), another clade between *A. punctata* from the eastern Atlantic (only those from Azores, Portugal) and a specimen from southwest Turkey; a few substitutions are also observed in specimens of *A. punctata* collected by us, together with specimens of the same species found both in the Mediterranean and Eastern Atlantic; the haplotype network shows a clear geographic structure in specimens of *A. punctata* from the Mediterranean, those from the eastern Atlantic and all the specimens collected by us (APJ and APA) in two well-defined groups: APJ7 which includes the specimens collected by us on the Catalonian coast such as APJ4, APJ5, APJ11, APJ12, APA2 and APA3; together with *A. punctata* from the Mediterranean of France, Italy, Turkey, Greece and Croatia; and other *A. punctata* from the eastern Atlantic, North Sea and the Norwegian archipelago; the other group is *A. punctata* from Livorno, Italy, which groups *A. punctata* from the Mediterranean of Spain, Greece and Turkey, with *A. punctata* from the eastern Atlantic of the North Sea and the APJ6 collected by us on the Catalonian coast (Fig. 3).

Species delimitation

The species recovered in the ASAP species delimitation analysis correspond to well-supported clades that include four different hypothetical “groups” of which it stands out; group four: with all the APJ and the APA collected by us together with all the *A. punctata* of the Mediterranean and the great majority of the eastern Atlantic (represented with the red bar); the other groups are constituted as follows: group one with the *A. fasciata* from the western Atlantic and Israel (Green Bar), group two: the *A. nigrocincta* from the Indo-Pacific and finally group three: the *A. punctata* from the eastern Atlantic (only those of Azores, Portugal) together with a specimen from southwest Turkey (blue bar) (Fig. 2).

Haplotype network

The COI dataset included 668 bases in the species *A. punctata*. The haplotype network showed five different haplotypes, differentiated by several substitutions from each other. The *A. punctata* clade has a haplotype that differs by many substitutions from the eastern Atlantic (only those from Azores, Portugal), and one specimen from southwest Turkey; a few substitutions are also observed in specimens of *A. punctata* collected by us, together with specimens of the same species found both in the Mediterranean and Eastern Atlantic; the haplotype network shows a clear geographic structure in specimens of *A. punctata* from the Mediterranean, those from the eastern Atlantic and all the specimens collected by us (APJ and APA) in two well-defined groups: APJ7 which includes the specimens collected by us on the Catalonian coast such as APJ4, APJ5, APJ11, APJ12, APA2 and APA3; together with *A. punctata* from the Mediterranean of France, Italy, Turkey, Greece and Croatia; and other *A. punctata* from the eastern Atlantic, North Sea and the Norwegian archipelago; the other group is *A. punctata* from Livorno, Italy, which groups *A. punctata* from the Mediterranean of Spain, Greece and Turkey, with *A. punctata* from the eastern Atlantic of the North Sea and the APJ6 collected by us on the Catalonian coast (Fig. 3).

External morphology

The smallest specimens (APJ), about 4-20 mm long, have a reddish-brown coloration due to a dense crosslinking of this color that delimits polygonal areas in the center, where there is usually an irregular white spot; also, we found some juveniles of length between 20–25 mm of brown coloration with white spots and morphology very similar to the adult specimens of *A. punctata* (Fig. 4). There are also usually larger white spots scattered along the lateral walls of the body and parapodium, which may be grouped into larger whitish spots. When the animal is moving, the body is elongated and slender and the head is joined to the rest of the visceral mass by a long neck, which can be shrunk and stretched. To the right of the body, the spermatic sulcus is clearly visible, which runs...
Fig. 2: Phylogenetic tree from the obtained sequences and those of Genbak. Consensus tree topology of mtDNA haplotypes and ASAP species delineation colored clusters; the specimens placed by us (APJ and APA), are in blue.
**Fig. 3:** Haplotype networks based on COI sequence data, the geographic region of origin of the specimens is represented in color. The relative size of the circles is proportional to the number of sequences of the same haplotype.

**Fig. 4:** Comparison between A and C. the “typical” small reddish APJ specimens with a length between 5-20 mm (cited as *A. parvula* in the Mediterranean), B. with juvenile *A. punctata* in the intermediate process to pass to the adult phase with a length between 20-25 mm and D. APA adult specimens with a length between 25-100 mm.
from the anterior end of the parapodium to almost the base of the right cephalic tentacle. The cephalic tentacles are curled throughout and the rhinophores have a slit in their lateral area; both are the same color as the body except for its apical area, which is usually black. The black eyes are located in a slightly prominent whitish areas somewhat to the right and in front of the base of the rhinophores. The mantle has a wide oval foramen through which the shell can be seen. The parapodia are not very wide, they are slightly undulating and they are fused behind. The edge of each parapodium is black, sometimes interrupted by white spots and in some specimens a fine white marginal line is observed. The parapodia are joined in the posterior end, which prevents the animal from making swimming movements. On the dorsal inside of the parapodium there are usually white granulations that form a spot of this very apparent color and that also adorn the anal siphon. The gill is pink and semitransparent. The foot is pink except in the anterior and posterior areas whose margins are black. There may also be white granulations on the rest of the foot margin (Fig. 5). The penis is spoon-shaped and pink and semi-transparent.

Above ~ 25 mm in length up (APA), specimens are more or less dark olive green with numerous white marks throughout the body, including the rhinophores and the dorsal area of the foot, sometimes gathered in specific

Fig. 5: Specimens of APA 65 mm length (left) and APJ 20 mm length (right). Localities: A: Es Caials; B: La Fosca; C: Fòrum; D: Fòrum; E: Bau del Cap Falcó; F: La Fosca; G: Cap Falcó; H: Bau del Cap Falcó.
areas. In these larger specimens the black pigmentation of cephalic tentacles, rhinophores, margin of the parapodium and anterior and posterior borders of the foot is no longer observed. In some darker specimens a white line is very apparent on the edge of the parapodium. The largest specimens (10-15 cm) are usually brownish in color and the white spots can cover a large part of the body or be very small and widely dispersed. In some of the larger specimens the spots are cream-colored with a whitish central area. The foot is wide behind and acts like a suction cup.

The shells of the specimens of *A. punctata* (Fig. 6) measure 10 mm in a 25 mm living animal and 35 mm in a 65 mm specimen, they are oval, wider about half their length, with a curved posterior end, the right part adjacent to the protoconch is slightly concave.

**Radular and jaw structure**

The radular formula of a 20 mm APJ specimen collected alive in La Fosca, Palamos, Barcelona, NE Spain was 35×5.10.1.10.5. The central tooth has a geometric shape similar to a trapezium with a very elongated base, a central triangular cusp and several lateral protuberances without clearly defined denticles, the teeth measure on average 54.91 wide × 111.95 µm long; the lateral teeth are narrow and have a main cusp with an inner lateral denticle and 2-3 denticles on the outer side; the outermost teeth are undeveloped, have an outer margin and an inner margin with a well-defined stalk, 79.49 µm long × 12.34 µm wide (Fig. 7 A, B and C right).

In a 85 mm APA specimen collected alive at the Forum, Barcelona, NE Spain the radular formula was 51×8.10.1.10.8. The central tooth has a trapezoidal geometric shape, with a serrated main cusp flanked with 2 denticles on each side; this rachidian tooth measures 94.22 µm long × 54.91 µm wide; lateral teeth are narrow and have a serrate main cusp, inner flanking cusp and reduced outer flanking cusp; they are 94.09 µm long × 48.56 µm wide; outermost they are 126.20 µm long × 16.99 µm wide (Fig. 7A, B and C left). The jaws of the APA and APJ specimens are formed by tiny curved and smooth hook-shaped rods called uncincas that serve to scrape food, having two to four cusps at the apex (Fig. 7D).

**Biology**

On the Catalonian coast, JPA grow up to a size of about 20 mm (Fig. 8B, C and D), preferentially live among the fronds of red soft algae such as *S. coronopifolius* (Fig. 8A) and *P. cartilagineum*. APJ specimens feed on different red algae and presumably obtain pigments that help them camouflage themselves to hide from predators. Specimens change from APJ (reddish) to APA (olive green) as they get larger.

Due to their larger size, APA they move to a different habitat, living under the rocks; at the same time, they begin to ingest green algae of the genus Ulva Linnaeus (which includes *Enteromorpha* Link), preferably *Ulva lactuca* Linnaeus which is a very common green algae in the Mediterranean; their bodies change from reddish to an olive-green hue with white spots, as they probably acquire pigments from their food and incorporate them into their tissue. Therefore, APA specimens acquire other green or brown tones depending on the algae available to feed on, in addition to the typical white spots that characterize the species. When specimens are disturbed, they can secrete a defensive whitish fluid, sometimes mixed with purple ink (Fig. 8C).

APJ and APA specimens reproduce at the end of spring (April-June), both in the field and in the laboratory, forming aggregations of up to 9-10 individuals (APA) and 4–5 individuals (APJ) (Fig. 9). During this period numerous APA specimens migrate to shallow depths to copulate and lay eggs. The breeding period is simultaneous in various locations such as Cubelles, Cadaqués and Fòrum de Barcelona; in this last place we have counted up to more than 150 APA specimens in one hour of sampling copulating and depositing their egg masses under stones at a depth of about 2 m. After copulation, the animals lay a row of orange-yellow eggs (Fig. 10) sometimes reddish to pink and sometimes mauve or orange, forming a compact mass. Inside the ribbon are capsules containing 3 or 4 eggs, each about 100 µm in diameter. Only APAs mate and lay eggs; APJs only copulate, but do not lay eggs (Fig. 11). In the last 40 years, on the Catalanian coast, 366 observations, 225 copulations and 92 APA clutches, 182 observations and two APJ mating events were recorded (Fig. 11, Table 3).

**Fig. 6:** Shells of specimens. A: APJ (A3). Collected in La Fosca, Barcelona. B: APA (Ap3) from El Fòrum, Barcelona.
Fig. 7: Scanning electron micrographs of the radular teeth and jaw elements of APA (left) from Fòrum (Barcelona, NE Spain) and APJ (right) from La Fosca (Palamós, NE Spain). A. Rachidian and first lateral teeth, B. lateral teeth, C. marginal and external lateral teeth D. uncinas of the jaw.
Fig. 8: A: the red algae *Sphaerococcus coronopifolius*; B, C and D: APJ of 20 mm length (known until recently as *A. parvula*) on the same alga, note the black margins of oral tentacles, rhinophores, parapodia, and anterior and posterior end of the foot; in C a specimen emitting the defensive violaceous substance after being disturbed.

Fig. 9: Reproduction in *Aplysia punctata*. A and B: multiple copulas in APA of 65 mm length; C and D: multiple copulas in APJ of 20 mm length (known until recently as *A. parvula*).
Discussion

The morphological differences between APJ and APA in body color (Fig. 5), shell (Fig. 6), and radular and jaw morphology (Fig. 7) examined herein suggest that they could be different species, and even that APJ could be a possible new species of *Aplysia*. However, the results of the molecular analyses, including the phylogenetic tree, the species delimitation analysis with the ASAP algorithm (Fig. 3), and the haplotype network (Fig. 4), clearly show that all the specimens from the Catalonian coast of APJ and APA belong to a single species that corresponds to *A. punctata*. Although in Figure 3 there is divergence in the phylogenetic results between the intraspecific genetic biodiversity of some *A. punctata* collected by us with others from the eastern Atlantic (Azores, Portugal), due to the few substitutions observed among these specimens (Fig. 4), genetically they are all still *A. punctata* (both APJ and APA) from the Mediterranean and the eastern Atlantic.

Our data agree with Golestani *et al.* (2019) in that there is no molecular evidence of the presence of *A. parvula* in the Mediterranean and in the eastern Atlantic, and the phenotypical resemblance of the APJ to the adults of *A. parvula* is possibly due to a protective mechanism of APJ from predators, to be able to hide better in red algae.

Regarding interspecific diversity, it is very evident in Figure 3 that the APJ, APA and the other *A. puncta-
ta from the Mediterranean and the Eastern Atlantic are very different from A. fasciata of the Eastern Atlantic, A. nigrocincta from the Indo-Pacific, and A. punctata recorded in Azores, Portugal; this can also be seen in Figure 4, where numerous substitutions occur between the APJ, APA and the other A. punctata from the Mediterranean and the Eastern Atlantic with A. fasciata from the Eastern Atlantic, A. nigrocincta from the Indo-Pacific, including A. punctata recorded in Azores, Portugal; the latter according to this paper and Golestani et al. (2019) constitute a different species, despite the fact that in our paper we found an A. punctata from southwest Turkey within the clade of A. punctata from Azores, Portugal (Fig. 3). Although all specimens from southwest Turkey were mistakenly recorded by Yokes (unpublished work) as A. parvula, the paper of Golestani et al. (2019), cites all specimens from southwest Turkey as A. punctata.

Garstang (1890) and Eales (1921) found that A. punctata in the Mediterranean feeds on eight species of algae, red algae in the juvenile stage and green algae as adults. On the other hand, Niell (1977) pointed out that A. punctata can feed on up to 32 species of red and green algae in the Atlantic Ocean, but almost always prefers Ulva gigantea Bland and Eales (1921) indicated that this species prefers Fucus cartilagineus Linnaeus and species of the genera Delesseria J.V. Lamouroux, Laminaria J.V. Lamouroux, and Ulva Linnaeus. Carefoot (1967a) found that juveniles of A. punctata in the British Islands feed preferentially on red algae of different genera, such as Plocamium Lamouroux, Heterosiphonia Montagne, Cryptopleura Kützing, and Delesseria Lamouroux, (in that order) when available. Also, Carefoot (1967b) used a combination of these red algae in the laboratory to feed specimens of A. punctata and found that they used the energy obtained by ingesting these algae for growth, nitrogen production, carbohydrates, and amino acid synthesis, and that they grew faster feeding on the species P. cartilagineus (as Plocamium coccineum Lyngbye).

In this paper, we have observed that APJ on the Catalan coast feed on a great variety of types of red algae, such as S. coronopifolius and P. cartilagineum, acquiring a reddish hue phenotypically similar to the adults of A. parvula due to the fact that they incorporate the pigments of the algae into their body. We hypothesize that as they increase in size, they no longer go unnoticed by predators in the red algae and have to implement alternative defensive strategies. One of such strategies is to feed on the green alga U. lactuca, which is available in their environment, and as they intake pigments from the algae, their body Changes to an olive-green hue with many white spots, becoming APA. They also move to a different habitat that may provide better protection, such as under rocks. These APA specimens acquire other green or brown tones, in addition to the typical white spots that characterize the species in its adult state, which help it to go unnoticed by predators, contributing to the ecological success of the species, which is very common in the Mediterranean.

Lo Bianco (1909) found that in the Mediterranean A. punctata reproduces from February to July, while Eales (1921) indicated that the reproductive period is from spring to summer, Miller (1960) from March to August, Carefoot (1967a) from May to October and Thompson (1976) in spring; all of them for the specimens of the Atlantic. We found Mediterranean APA copulating and laying eggs, and APJ only copulating in April and June, but not copulating with each other, forming reproductive aggregates of 9-10 individuals of APA (Figs 9A, 9B) and 4-5 individuals of red APJ (Figs 9C, 9D), confirming that APJ specimens are sexually mature and therefore smaller adults. Carefoot (1987) observed breeding APA groups of 22 to 30 individuals.

Ballesteros and Templado (1987) indicated that the eggs in APA are laid forming a ribbon of 0.6 mm in diameter with abundant reddish or pinkish eggs, each with a diameter between 80 and 105 μm. We found that the egg masses vary in color depending on the size of the animal, probably a defensive mechanism against predators. Our APA specimens produced pink or reddish egg masses, very similar in color to the algae they feed on (Fig. 10). They also produce egg masses that vary from yellow to pink, and also mauve (Carefoot, 1967b) or orange (Eales, 1921), the latter coinciding with those observed in this study; APJs do not produce egg masses, they were only observed copulating (Fig. 11), because despite maturing early, they may not be able to fertilize their eggs.

In conclusion, based on the results of this study, it appears that changes in the diet of A. punctata determine body color, providing this species with a dynamic, adaptable defensive mechanism that would explain the dominance of this species in the Mediterranean Sea.

Acknowledgements

We are grateful to the IFARHU-SENACYT Scholarship Program for the financial support and the Universities of Panama and Barcelona. This work has been financed thanks to the research funds of the Grup de Recerca Consolidat en Biologia i Ecologia Bentòniques of the University of Barcelona to which Dario Córdoba and Manuel Ballesteros belong. Our thanks to Carles Gaillà, Andrea Cabrito, Oriol Castells and Helena Baños who have accompanied us in the samplings along the Catalan coast or helped in the molecular genetics laboratory. Miquel Pontes has provided us with numerous citations of APJ (as A. parvula) and APA on the Catalan coast that are in his data base.

References

Andrews, K.R., Norton, E.L., Fernández-Silva, I., Portner, E., Goetze, E. et al., 2014. Multilocus evidence for globally distributed cryptic species and distinct populations across ocean gyres in a mesopelagic copepod. Molecular Ecology, 23, 5462-5479.

Ballesteros, M., 2007. Lista actualizada de los opistobranquios (Mollusca: Gastropoda: Opisthobranchia) de las costas catalanas. Spira, 2 (3), 163-188.
Ballesteros, M., Templado, J., 1987. *Aplysia parvula* Guilding in Mörch, 1863 en las costas de la península ibérica. *Publicaciones del Departamento de Zoología*, 13, 55-62.

Ballesteros, M., Madrenas, E., Pontes, M., 2016. Actualización del catálogo de los moluscos opistobranquios (Gastropoda, Heterobranchia) de las costas catalanas. *Spira*, 6, 1-28.

Ballesteros, M., Madrenas, E., Pontes, M., 2021. OPK Opistobranquis. https://opistobranquis.info/en/FlmKM (Accessed 26 November 2021).

Bouchet, P., Gofas, S., 2022. *World Register of Marine Species* http://www.marinespecies.org/aphia.php?p=taxdetails&id=137654 (Accessed 14 January 2022).

Carefoot, T.H., 1967a. Growth and nutrition of *Aplysia punctata* feeding on a variety of marine algae. *Journal of the Marine Biological Association of the U.K.*, 47, 565-589.

Carefoot, T.H., 1967b. Studies on a sublittoral population of *Aplysia punctata*. *Journal of the Marine Biological Association of the U.K.*, 47, 335-350.

Carefoot, T.H., 1987. *Aplysia*: its biology and ecology. *Oceanography and Marine Biology: Annual Review*, 25, 167-284.

Carmona L., Lei B.R., Pola M., Gosliner T.M., Valdés A. et al., 2014. Untangling the *Spurilla* neapolitana (Delle Chiaje, 1841) species complex: a review of the genus *Spurilla* Bergh, 1864 (Mollusca: Nudibranchia: Aeolidiidae). *Zoological Journal of the Linnean Society*, 170 (1), 132-154.

Cervera, J.L., Calado, G., Gavaia, C., Malaquias, M.A.E., Templado, J. et al., 2004. An annotated and updated checklist of the opistobranchs (Mollusca: Gastropoda) from Spain and Portugal (including islands and archipelagos). *Boletín del Instituto Espanol de Oceanografía*, 20, 1-111.

Clement, M., Posada, D., Crandall, K.A., 2000. Graphicraft Limited, Hong Kong TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657-1659.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F. et al., 2010. The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS ONE*, 5 (8), e11842.

Cooke, S., Hanson, D., Hirano, Y., Oremelas-Gatdula, E., Gosliner, T.M. et al., 2014. Cryptic diversity of *Melanocholemys* sea slugs (Gastropoda, Aglaieidae) in the North Pacific. *Zoologica Scripta*, 43, 351-369.

Corsini-Foka, M., Zenetos, A., Crocetta, F., Çinar, M.E., Koçak, F. et al., 2015. Inventory of alien and cryptogenic species of the Dodecanese (Aegean Sea, Greece): collaboration through COST Action training school. *Management of Biological Invasions*, 6 (4), 351-366.

Crocetta, F., Poursanidis, D., Tringali, L.P., 2015. Biodiversity of sea slugs and shelled relatives (Mollusca: Gastropoda) of the Cretan Archipelago (Greece), with taxonomic remarks on selected species. *Quaternary International*, 390, 56-68.

Domènech, A., Avila, C., Ballesteros, M., 2002. Spatial and Temporal variability of the opisthobranch molluscs of Port Lligat Bay (Catalonia, NE Spain). *Journal of Molluscan Studies*, 68, 29-37.

Doyle, J.J., 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany*, 17, 144-163.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A. et al., 2010. *Geneious v5.1*. http://www.geneious.com.

Eales, N.B., 1921. *Aplysia*. *L.M.B.C. Memoirs on Typical British Marine Plants and Animals*, 24, 84.

Field, K.G., Olsen, J.J., Lane, D.J., Giovannoni, S.J., Ghiselin, M.T. et al., 1988. Molecular phylogeny of the animal kingdom. *Science*, 239 (4841 Pt 1), 748-53.

Fukami, H., Budd, A.F., Paulay, G., Solé-Cava, A., Chen, C.A. et al., 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature*, 427, 832.

Gaither, M.R., Bowen, B.W., Rocha, L.A., Briggs, J.C., 2016. Fishes that rule the world: circumtropical distributions revisited. *Fish and Fisheries*, 17, 664-679.

Garstang, W., 1890. A complete list of the opisthobranchiate Mollusca found at Plymouth; with further observations on their morphology, colours, and natural history. *Journal of the marine biological Association of the U.K.*, 1, 399-457.

Gofas, S., Luque, A.A., Templado, J., Salas, C., 2017. A national checklist of marine Mollusca in Spanish waters. *Scientia Marina*, 81 (2), 241-254.

Golestani, H., Crocetta, F., Padula, V., Camacho-Garcia, Y., Langeneck, J. et al., 2019. The little *Aplysia* coming of age: from one species to a complex of species complexes in *Aplysia parvula* (Mollusca: Gastropoda: Heterobranchia). *Zoological Journal of the Linnean Society*, 187 (2), 279-330.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analyses. *Systematic Biology*, 42, 182-192.

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.

Kienberger, K., Carmona, L., Pola, M., Padula, V., Gosliner, T.M. et al., 2016. *Aeolidia papillosa* (Linnaeus, 1761) (Mollusca: Heterobranchia: Nudibranchia), single species or a cryptic species complex? A morphological and molecular study. *Zoological Journal of the Linnean Society*, 177, 481-506.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-20.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. p. 73-90. In: *Marine genetics*. Solé-Cava, A.M., Russo, C.A.M., Thorpe, J.P. (Eds). Springer, Dordrecht.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger data-sets. *Molecular Biology and Evolution*, 33, 1870-1874.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B. et al., 2017. Partition Finder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution*, 34 (3), 772-773.

Leigh, J.W., Bryant, D., 2015. PopART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110-1116.

Lindsay, T., Valdés, Á., 2016. The model *Hermissenda crassicornis* (Gastropoda: Heterobranchia) is a species complex. *PLoS One*, 11, e0154265.

Lo Bianco, S., 1909. Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animale del
golfo di Napoli. Mittheilungen aus der Zoologischen Station zu Neapel, 19, 513-761.

Martín, D., Dantart, L., Ballesteros, M., 1990. Moluscos de las concreciones de algas calcáreas del litoral catalán (NE España). Lavori S.I.M., 23, 445-456.

Medina, M., Walsh, P.J., 2000. Molecular systematics of the order Anaspidea based on mitochondrial DNA sequence (12S, 16S, and COI). Molecular Phylogenetic and Evolution, 15, 41-58.

Medina, M., Collins, T., Walsh, P., 2001. mtDNA Ribosomal Gene Phylogeny of Sea Hares in the Genus Aplysia (Gastropoda, Opisthobranchia, Anaspidea): Implications for Comparative Neurobiology. Systematic Biology, 50(5), 676-688.

Miller, M.C., 1960. A note on the life history of Aplysia punctata Cuvier in Manx waters. Proceedings of the malacological Society of London, 34, 165-167.

Niell, F.X., 1977. L’alimentation d’Aplysia punctata (Gastropoda, Opisthobranchia) Cuvier dans la Ría de Vigo (Galice). I. Analyse du contenu digestif d’individus de la zone intertidale. Malacologia, 16 (1), 207-209.

Ornelas-Gatdula, E., Camacho-García, Y., Schrödl, M., Padula, V., Hooker, Y. et al., 2012. Molecular systematics of the ‘Navanax aenigmaticus’ species complex (Mollusca, Cephalaspidea): coming full circle. Zoologica Scripta, 41, 374-385.

Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. Molecular Ecology Resources, 21, 609-620.

Ribero, N., Martínez, R., Pauls, S., 1998. Primer registro de Aplysia (Pruvotaplysia) parvula Mörch, 1863 (Mollusca, Opisthobranchia, Aplysiidae) para Venezuela. Acta Biologica Venezolana, 18 (1), 43-47.

Sánchez Tocino, L., 2001. Opistobranquios de la Costa de Granada. http://www.ugr.es/~lstocino/aplysia_parvula.htm (Accessed 14 December 2014).

Stamatakis, A., 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics, 30 (9), 1312-3.

Thompson, T.E., 1976. Biology of Opisthobranch Molluscs. Ray Society, London, 207 pp.

Uribe, R., Sepúlveda, F., Goddard, J.H.R., Valdés, Á., 2018. Integrative systematics of the genus Limacia O. F. Müller, 1781 (Mollusca, Gastropoda, Nudibranchia, Polyceridae) in the Eastern Pacific. Marine Biodiversity, 48 (4), 1815-1832.

Valdés, Á., Breslau, E., Padula, V., Schrödl, M., Camacho-García, Y. et al., 2017. Molecular and morphological systematics of Dolabrifera Gray, 1847 (Mollusca: Gastropoda: Heterobranchia: Aplysiomorpha). Zoological Journal of the Linnean Society, 184 (1), 31-65.

Zenetos, A., Mačić, V., Jaklin, A., Lipaj, L., Poursanidis, D. et al., 2016. Adriatic ‘opisthobranchs’ (Gastropoda, Heterobranchia): shedding light on biodiversity issues. Marine Ecology - An evolutionary perspective, 37 (6), 1239-1255.