

***Doris adrianae* sp. nov. (Heterobranchia; Nudibranchia; Doridina)  
from the Galician coasts (NW Iberian Peninsula)**

***Doris adrianae* sp. nov. (Heterobranchia; Nudibranchia; Doridina)  
de las costas de Galicia (NW Península Ibérica)**

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### Abstract

A new species of dorid nudibranch, *Doris adrianae* sp. nov. is described, from the Ría de Ferrol (NW Iberian Peninsula) on rocky bottoms, between 11 and 20 m deep, where its prey, the sponge *Polymastia boletiformis* (Lamarck, 1815) is common. The new species is oval-shaped and yellow to yellow-orange in colour, with the dorsum covered by rounded tubercles of various sizes, reinforced by tegumentary spicules. This new species is characterised by having numerous integumentary and fusiform calcareous spicules, mainly grouped in multispicular bundles resulting in a complex and very dense skeletal structure, giving the animal great consistence without losing flexibility. In addition, it differs from other known species of the genus *Doris* Linnaeus, 1758 by various external and internal characters, mainly by the coloration and the shape of the tubercles and the morphology of the radula, the digestive and reproductive systems. *Doris adrianae* sp. nov. also presents a marked genetic distance in the barcode fragment (*cox1-5'*) with other species of the genus *Doris*.

**Keywords:** Gastropoda, Ría de Ferrol, anatomy, habitat, feeding, micro computed tomography.

### Resumen

Se describe una nueva especie de un nudibranquio doridáceo, *Doris adrianae* sp. nov., recolectada en la Ría de Ferrol (NW Península Ibérica) en fondos de roca, entre 11 y 20 m de profundidad, donde es frecuente el porífero *Polymastia boletiformis* (Lamarck, 1815) del que se alimenta. La nueva especie tiene forma ovalada y de color amarillo a amarillo-anaranjado, con el dorso cubierto por tubérculos redondeados de varios tamaños, reforzados por espículas tegumentarias. Se caracteriza por presentar numerosas espículas calcáreas tegumentarias fusiformes, principalmente agrupadas en haces multiespiculares que le confieren una estructura esquelética compleja y muy densa, dándole al animal una gran consistencia sin perder flexibilidad. Además, se diferencia de otras especies conocidas del género *Doris* Linnaeus, 1758 por diversos caracteres externos e internos, principalmente por la coloración y tubérculos del cuerpo, caracteres de la rádula y de los aparatos

digestivo y reproductor. *Doris adrianae* sp. nov. también presenta una marcada distancia genética en el fragmento “barcode” (*cox1-5'*) con otras especies del género *Doris*.

**Palabras clave:** Gastropoda, Ría de Ferrol, anatomía, hábitat, alimentación, microtomografía computarizada.

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## Abbreviations

a: anus	megl: membrane gland
am: ampulla	mugl: mucous gland
ao: aorta	mot: retractor muscles
ar: adhesive region	og: gastro-oesophageal ganglion
au: auricle	ot: oral tube
ba: anterior blood gland	ovp: proximal oviduct
bb: buccal bulb	ovd: distal oviduct
bc: bursa copulatrix	p: pedal nerves
bg: buccal ganglion	pc: pedal commissure
bp: posterior blood gland	pe: penis
c: cerebral nerves	pg: pedal ganglion
ca: caecum	pl: pleural nerves
cg: cerebral ganglion	plg: pleural ganglion
cgl: capsule gland	ppc: parapedal commissure
d: deferent duct	pr: prostate
dg: digestive gland	ps: penial sheath
e: oesophagus	pu: pericardium
ey: eye	r: rhinophoral nerves
fg: female glands	rs: radular sac
g: branchial leaves	sg: salivary gland
ga: genital atrium	sr: seminal receptacle
go: genital openings	st: stomach
gpo: postampullar gonoduct	sy: syrinx
gpr: preampullar gonoduct	ud: uterine duct
hdg: hermaphrodite and digestive glands	va: vagina
hg: hermaphrodite gland	ve: ventricle
i: intestine	vgl: vestibular gland
m: mouth	vl: visceral loop
mbb: buccal bulb muscle	

## INTRODUCTION

The coasts of the Iberian Peninsula represent a zoogeographic enclave comprising two regions: Lusitanian and Mediterranean (GOFAS, 2011). A total of 715 species of heterobranchs sea slugs are known from the Iberian Peninsula and the Balearic and

Canary Islands, of which 279 belong to the order Nudibranchia (GOFAS, *et al.* 2017; CERVERA, *et al.* 2004). Galicia (NW Iberian Peninsula) represent more than a third of the total coastline of the Iberian Peninsula, as it is heavily indented, with exposed environments

open to the beating of the ocean and with deep coastal indentations called rías (RICHTHOFEN, 1886). The latter were formed by marine flooding of a terminal river course caused by the subsidence of the coastal edge and the rise in sea level. The rías vary greatly in size and orography, which determines the existence of a great diversity of habitats, from very rough to very sheltered environments, with a high diversity of flora and fauna (BAÑÓN, 2017).

The heterobranchs sea slugs of Galicia were poorly known until the last quarter of the 20th century, when URGORRI (1981) wrote his doctoral thesis and the first records and new species were published (ORTEA & URGORRI, 1978, 1979a, 1979b, 1981a, 1981b; among others). URGORRI & BESTEIRO (1983) published the inventory of the “opisthobranchs” of Galicia, in which they compiled the data from the doctoral thesis of URGORRI (1981) and all previous data, cataloguing 148 species from coastal and bathyal bottoms down to 3000 m depth. In subsequent years, the inventory was increased with new species and records, mainly of nudibranchs, by various authors (see URGORRI, *et al.* 2011). Currently, 110 species of the order Nudibranchia are known from Galicia, of which 62 are classified in the suborder Cladobranchia and 48 in the suborder Doridina (TRIGO, *et al.* 2018).

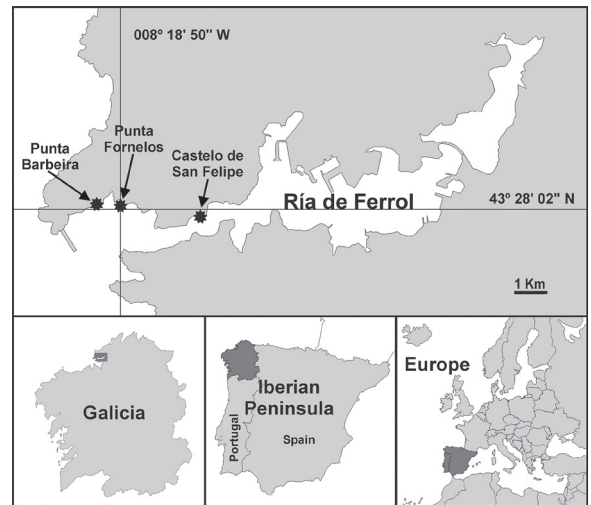
Three species of the genus *Doris* Linnaeus, 1758 are known in Galicia. These include *Doris verrucosa* Linnaeus, 1758 (URGORRI & BESTEIRO, 1983, 1984; ROLÁN, 1983; TRIGO & OTERO, 1987; TRIGO, *et al.* 2018). *Doris pseudoargus* Rapp, 1827 (URGORRI & BESTEIRO, 1983, 1984, both as *Archidoris pseudoargus*; ROLÁN (1983), as *A. pseudoragus*; TRIGO, *et al.* 2018) and *Doris ocelligera* (Bergh, 1881 in BERGH 1881a) (URGORRI & BESTEIRO, 1983, 1984; ROLÁN, 1983; TRIGO, *et al.* 2018). All three species are well known from the Ría de Ferrol and found on rocky and gravel substrata in both the lower mesolittoral and infralittoral.

Twenty-two specimens of a dorid were collected at the Ría de Ferrol during the last 34 years, between 1986 and 2019, on a rocky bottom between 11 and 20 m deep, mostly under a gorgonian forest of *Leptogorgia*

*lusitanica* (Stiasny, 1937). After its study, it was classified within the genus *Doris* according to VALDÉS (2002) and was found to be a new species described here as *Doris adrianae* sp. nov.

## MATERIAL AND METHODS

**Collection:** The 22 specimens studied were collected by SCUBA diving, from rocky bottoms between 11 and 20 m deep in the localities of Punta Fornelos, Castelo de San Felipe and Punta Barbeira, at the Ría de Ferrol (NW Iberian Peninsula) (Fig. 1).



**Figure 1.** Map showing the type locality of *Doris adrianae* sp. nov. in Punta Fornelos, Castelo de San Felipe and Punta Barbeira at the Ría de Ferrol (NW Iberian Peninsula) where all specimens were collected.

**Figura 1.** Mapa que muestra la localidad tipo de *Doris adrianae* sp. nov. en Punta Fornelos, Castelo de San Felipe y Punta Barbeira en la Ría de Ferrol (NW Península Ibérica) donde se recolectaron todos los ejemplares.

They were collected mostly one specimen per sampling; only 6 times 2 specimens were collected together. In some samplings, *in situ* photographs of the animal, habitat and accompanying species were taken. Samples of porifera that could potentially be preyed upon by the dorid were also collected, in the same habitat. All specimens were observed *in vivo* and all characters that were lost after fixation were described. Some specimens were photographed *in vivo* in their habitat, although most were photographed in the laboratory. Both the general appearance of the animal and details of its external anatomy were captured, using

an Olympus SCX12 stereo microscope with an Olympus E-330 digital camera. In addition, the animals were kept alive for 2 or 3 days in individualised aquaria for the collection of faeces and spawning.

**Anaesthesia, fixation and preservation:** All specimens were anaesthetised with 7% magnesium chloride (MgCl<sub>2</sub>), diluted in equal parts (50%) in seawater and fresh water (URGORRI, 1981). After anaesthetising, the fixation process was carried out gradually, adding drops of the diluted fixative liquid with a Pasteur pipette, observing under the stereoscopic microscope if any reaction was produced in the animal, until complete fixation. The fixative liquid used was 70% EtOH in 7 specimens, 100% EtOH in 6 specimens and Bouin's fluid in 10 specimens; specimens fixed in 70% EtOH and Bouin's fluid were preserved in 70% EtOH neutralized with Borax to avoid degradation of the calcareous structures, while those fixed in EtOH 100° were preserved in EtOH 100° (for details see Type material and deposition).

**Dissection, radula and labial disc preparations:** For the study of the internal anatomy, paratypes 1, 7, 10, 12 and 17 were dissected under an Olympus SCX12 stereo microscope with an Olympus E-330 digital camera. After dissection, preparations of the radula and labial disc were made for light microscopy (LM) and scanning electron microscopy (SEM), by macerating the buccal bulb with 5% KOH. For LM the radula and labial disc were mounted in Hoyer's liquid, while for SEM, the radula was mounted in distilled water between two coverslips and the labial disc in water in a small container, frozen in liquid nitrogen and lyophilised; Thus the radula was perfectly stretched and the lip disc was not wrinkled, glued to the SEM stub with DHMF resin (dimethyl-hydantoin formaldehyde), sputter coated with gold-palladium on a Bio-Rad e5000 coated and examined under a LEO-435VP SEM with Microanalysis (EDX, Oxford 300) Leica Microsystems (Cambridge, U. K.).

**Spicule preparations:** The study of the spicules and spicular skeletal arrangement presented numerous problems, as the traditional methods of isolation: 4% NaOH, 4% KOH,

5% NaClOH and H<sub>2</sub>O<sub>2</sub>, resulted in spicules decay. Thus, alternative methods were used: Cl<sub>2</sub>CH<sub>4</sub>, CaO, 0'25% trypsin, protease K at 15 ppm, natural bacterial decomposition in seawater and incineration of the organic matter in a muffle at 500°C for 48 hours, the latter being the one with which the best results were obtained. Spicule preparations for OM were mounted in Hoyer's liquid, synthetic resin and Canada balsam, while for SEM they were mounted on a circular glass coverslip glued with a carbon adhesive to the SEM stub, sputter coated with gold-palladium. The study of the skeletal arrangement was performed by X-ray computed microtomography (Micro-CT Skyscan 1172).

**Serial sectioning:** Serial sections 15 µm thick were taken from two specimens (Paratypes 4 and 15 and from parts of the dissection of paratypes 10 and 17) which were fixed in Bouin's fluid, decalcified with ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, sectioned with a Microm HM310 rotary microtome and stained with Mallory's trichrome (GIL-MANSILLA, *et al.* 2008).

**Micro-CT:** The study of the anatomical and skeletal structure was carried out using micro-computed tomography, the specimens used (Paratypes 6 and 16) were preserved in 70% ethanol. Subsequently, they were dehydrated in successive baths of 80%, 90% and 96% ethanol. The specimens were scanned with a Skyscan 1172 microtomograph.

Paratype 6 was scanned three times with the following treatments and parameters:

1.- The specimen was stained with 1% iodine in 96° ethanol during one week before dehydration with hexamethyldisilazane (HMDS) for four hours, and subsequently air dried overnight (ALBA-TERCEDOR & SANCHEZ-TOCINO, 2011; FAULWETTER, *et al.* 2013; CANDÁS, *et al.* 2016). The following parameters were used: 55 kv, 165 µA, no filter and pixel size of 6.78 µm. The sample was rotated 360° and the projection images were obtained at 0.25° intervals.

2.- The specimen was placed inside a closed polypropylene tube with a paper moistened with 96° EtOH at the bottom to maintain a moist atmosphere inside. The scanning parameters

were: 55 kv, 165  $\mu$ A, no filter and pixel size of 13.57  $\mu$ m. The sample was rotated 360° and the projection images were obtained at 0.30° intervals.

3.- The specimen was submerged in 5.5 % EDTA in 10% formaldehyde for four days. Then it was dehydrated in successive baths of 80%, 90% and 96% ethanol. Subsequently, it was stained again with 1% iodine in 96° ethanol during five days before dehydration with HMDS for four hours, prior to scanning. Scanning was performed at: 55 kv, 165  $\mu$ A, no filter and pixel size of 13.57  $\mu$ m. The sample was rotated 360° and the projection images were obtained at 0.20° intervals.

Paratype 16 was scanned twice with the following treatments and parameters:

1.- The specimen was stained with 1% iodine in 96° ethanol during one week before scanning. It was placed inside a closed polypropylene tube with a paper moistened with 96° ethanol at the bottom to maintain a moist atmosphere inside. The scanning parameters were as follows: 60 kv, 167  $\mu$ A, no filter and pixel size of 13.57  $\mu$ m. It was rotated 360° and the projection images were obtained at 0.30° intervals.

2.- The specimen was stained with 1% iodine in 96° ethanol during two weeks before scanning. It was placed inside a closed polypropylene tube with a paper moistened with 96° ethanol at the bottom to maintain a moist atmosphere inside. The scanning parameters were as follows: 60 kv, 167  $\mu$ A, no filter and pixel size of 13.57  $\mu$ m. It was rotated 360° and the projection images were obtained at 0.25° intervals.

The images were reconstructed with the NRecon software (Bruker, Belgium) and the obtained sections were cleaned with the CTAnalyzer software (Bruker, Belgium). The software CTVox and DataViewer (Bruker, Belgium) were used for the correct visualization of the data. After studying the obtained sections, 3D reconstruction was carried out using the program AVIZO 6.4 (Thermo Fisher Scientific) which allows three-dimensional anatomical models to be obtained from the two-dimensional images of the sections. After choosing

the pixel size and loading the cross-sectional images into AVIZO, they were aligned, drawing the different structures manually in the form of overlapping colour layers and then smoothed, to eliminate imperfections in order to obtain a sharp three-dimensional image.

**DNA analysis:** For molecular analysis, a fragment of the foot of Paratype 20 was soaked in water for 30 minutes before genomic DNA extraction with DNeasy Blood & Tissue Kit (Qiagen, Germany). A 657 base pair region from the 5' end of mitochondrial *cox1* ("barcode region") was amplified with standard LCO / HCO primers (FOLMER, *et al.* 1994). Amplification was performed with Bioline-MyTaq and the following cycling: 94°C for 2 min 30 sec, 40 cycles of 94°C for 30 s, 47°C for 45s and 72°C for 1 min 15 sec, and final extension of 72°C for 10 min. PCR products were sent to StabVida (Portugal) for purification with magnetic beads and sequencing in both directions using ABI 3730xl DNA Analyzer. Sequence chromatograms were assembled and manually edited using Geneious 5.6.

A total of 177 *cox1-5'* sequences within the genera *Doris*, *Archidoris* and *Austrodoris* were downloaded from GenBank in December 2020. The following species were represented, according to GenBank identification: 1 sequence of *Archidoris montereyensis*, 1 sequence of *Archidoris pseudoargus*, 1 sequence of *Archidoris wellingtonensis*, 93 sequences of *Austrodoris kerguelenensis*, 64 sequences of *Doris kerguelenensis*; 9 sequences of *Doris montereyensis*, 1 sequence of *Doris nobilis*, 6 sequences of *Doris pseudoargus*, 1 sequence of *Doris* sp. (this sequence was discarded in further analysis as it was not identified to species level). The full list of downloaded sequences is provided in Supplementary material Appendix 1. Unique haplotypes were identified (n= 132; including the barcode sequence of *Doris adrianae* sp. nov.) and an alignment of 627 bp was produced with Muscle in Geneious 5.6 after sequence trimming. One sequence per species (according to GenBank identity) was selected and an uncorrected pairwise identity (i.e., proportion of identical sites) was computed as a distance matrix in

Geneious 5.6. This preliminary analysis confirmed that *Archidoris*, *Austrodoris* and *Doris* were synonymous genera. Thus, one sequence per non-synonymous species of *Doris* as well as an outgroup sequence (*Hexabranthus* sp., GenBank MN224071.1) were included in a phylogenetic tree run in Beast2.2.6 (BOUCKAERT *et al.* 2019). Model priors were set in BEAUti as a GTR + G + I and uncorrelated relaxed clock with mcmc = 10 000 000 and log every 1 000. In TreeAnnotator, 5000 (50%) trees were discarded and a maximum clade credibility tree with median heights was built.

## RESULTS

Class **Gasteropoda** Cuvier, 1795

Subclass **Heterobranchia** Burmeister, 1837

Orden **Nudibranchia** Cuvier, 1814 in Blainville, 1814

Suborden **Doridina** Bouchet *et al.*, 2017

Superfamily **Doridoidea** Rafinesque, 1815

Family **Dorididae** Rafinesque, 1815

Genus ***Doris*** Linnaeus, 1758

Type species: *Doris verrucosa* Linnaeus, 1758, by subsequent designation: BOUCHET & VALDÉS (2000).

### ***Doris adrianae* Urgorri & Señaris sp. nov.**

<http://zoobank.org/urn:lsid:zoobank.org:pub:51C800B8-6F45-4F56-8E68-ED-6D53ECF248>

**Diagnosis:** Oval-shaped and orange-yellow in colour with fine black dorsal stippling, denser in the mid-lateral areas, 32-76 mm long and 18-55 mm wide. Dorsum covered with rounded tubercles of various sizes, slightly pointed, with a rough surface and a small basal stalk. Lamellar rhinophores and 7-8 tripinnate branchial leaves, surrounded at their base by wide sheaths with small tubercles on their surface. Foot orange, frontally bilaminate without notch. Head with two lateral oral lobes and without tentacles. Radular formula 0:33-53:3-4 x 38-50. Rachidian tooth absent, lateral teeth simple and curved with prominent ridge, with first central lateral teeth strongly curved and marginal teeth with plumose edge. Labial disc peltate in shape with soft rugosities.

Gastric caecum near the opening to the digestive gland. Reproductive system triaulic, ampulla long, tubular and thick, penis fusiform and unarmed, prostate large and granular in appearance. Vagina short and wide, bursa copulatrix large and oval, three times larger than the oval seminal receptacle. Vestibular gland at the end of the distal oviduct, near its opening in the genital atrium.

**Derivatio nominis.** The species is dedicated to Adriana Álvarez Urgorri, granddaughter of the senior author.

**Type locality:** Ría de Ferrol (Galicia, NW Iberian Peninsula): Punta Fornelos (43° 28' 02" N; 008° 18' 50" W) all animals (Holotype and Paratype 1-16) were collected between 16 and 20 m deep on rocky bottoms with muddy sand sedimentation; Castelo de San Felipe (43° 27' 47" N; 008° 16' 57" W) all animals (Paratype 17-20) were collected between 11 and 18 m deep on rocky bottoms with sand on the surface; Punta Barbeira (43° 28' 3.19" N; 008° 19' 11.52" W) all animals (Paratype 21-22) were collected between 12 and 18 m deep on rocky bottoms with muddy sand sedimentation (Fig. 1).

**Other localities:** 01010204: 3 specimens and 01250404: 1 specimen in O Grelle (Ría da Coruña, NW Iberian Peninsula) (43° 22' 53" N; 008° 23' 30" W). The 4 specimens were not collected but were photographed *in situ*, on rocky bottoms with small red algae and incrusting sponges at a depth between 12-16 m. 01141190: 1 specimen 50 mm long at Sálvora (Ría de Arousa, NW Iberian Peninsula): (42° 25' 38" N; 009° 0.478' W). It was collected on maërl bottoms with *Veretillum cynomorium* (Pallas, 1766) and *Pteroeides griseum* (Bohadsch, 1761), at a depth of 60 m. Orig. fixative and preserved in 70% EtOH neutralized with Borax.

### **TYPE MATERIAL AND DEPOSITION:**

**Holotype:** 02020892 (MHN-USC 10119). 1 specimen, complete, 65 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 02/08/1992. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 1:** 01060786-01 (MHN-USC 10119-01). 1 specimen, dissected, 50 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 06/07/1986. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 2:** 01060786-02 (MHN-USC 10119-02). 1 specimen, complete, 40 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 06/07/1986. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 3:** 01160591 (MCUC.2021.1.1). 1 specimen, complete, 32 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 16/05/1991. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 4:** 02030592 (MHN-USC 10119-04). 1 specimen, histological sections, 48 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 03/05/1992. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax. The anterior and posterior portions of the specimen are still embedded in paraffin block.

**Paratype 5:** 02300596 (ZSM Mol 20210004). 1 specimen, complete, 37 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 30/05/1996. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 6:** 05050896 (MHN-USC 10119-06). 1 specimen, complete, 40 mm long *in vivo*, scanned in the Micro-CT. Punta Fornelos (Ría de Ferrol) 05/08/1996. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 7:** 05130597-01 (MHN-USC 10119-07). 1 specimen, dissected, 60 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 13/05/1997. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 8:** 05130597-02 (MHN-USC 10119-08). 1 specimen, complete, in 45 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 13/05/1997. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 9:** 01080598-01 (MCUC.2021.1.2). 1 specimen, complete, 50 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 08/05/1998. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 10:** 01080598-02 (MHN-USC 10119-10). 1 specimen, dissected, 45 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 08/05/1998. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 11:** 02101199 (MHN-USC 10119-11). 1 specimen, complete, 40 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 10/11/1999. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 12:** 01240505 (MHN-USC 10119-12). 1 specimen, dissected, 40 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 24/05/2005. Orig. fixed and preserved in 100% EtOH.

**Paratype 13:** 03220705 (MHN-USC 10119-13). 1 specimen, complete, 40 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 22/07/2005. Orig. fixed and preserved in 100% EtOH.

**Paratype 14:** 04190805 (MHN-USC 10119-14). 1 specimen, dissected, 50 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 19/08/2005. Orig. fixed and preserved in 70% EtOH neutralized with Borax.

**Paratype 15:** 03040106 (MHN-USC 10119-15). 1 specimen, histological sections, 51 mm long *in vivo*. Punta Fornelos (Ría de Ferrol) 04/01/2006. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 16:** 01240510 (MHN-USC 10119-16). 1 specimen, complete, 41 mm long *in vivo*, scanned in the Micro-CT. Punta Fornelos (Ría de Ferrol) 24/05/2010. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 17:** 01290806-01 (MHN-USC 10119-17). 1 specimen, dissected and cut in histological sections, 71 mm long *in vivo*. Castelo de San Felipe (Ría de Ferrol) 29/08/2006. Orig. fixed and preserved in 100% EtOH.

**Paratype 18:** 01290806-02 (MHN-USC 10119-18). 1 specimen, dissected, 63 mm long *in vivo*. Castelo de San Felipe (Ría de Ferrol) 29/08/2006. Orig. fixed and preserved in 100% EtOH. Spawning sample for DNA.

**Paratype 19:** 01220807-01 (MHN-USC 10119-19). 1 specimen, complete, 76 mm long *in vivo*. Castelo de San Felipe (Ría de Ferrol) 22/08/2007. Orig. fixed and preserved in 100% EtOH.

**Paratype 20:** 01220807-02 (MHN-USC 10119-20). 1 specimen, complete, 55 mm long *in vivo*. Castelo de San Felipe (Ría de Ferrol) 22/08/2007. Orig. fixed and preserved in 100% EtOH. Spawning sample for DNA.

**Paratype 21:** 01020519-01 (MHN-USC 10119-21). 1 specimen, complete, 40 mm long *in vivo*. Punta Barbeira (Ría de Ferrol) 02/05/2019. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

**Paratype 22:** 01020519-02 (MHN-USC 10119-22). 1 specimen, complete, 55 mm long *in vivo*. Punta Barbeira (Ría de Ferrol) 02/05/2019. Orig. fixed in Bouin's solution and preserved in 70% EtOH neutralized with Borax.

The Holotype and the Paratypes 1, 2, 4, 6, 7, 10-20 have been deposited at the Museo de Historia Natural of the Universidade de Santiago de Compostela, Galicia, Spain (MHN-USC). Paratypes 3 and 9 have been deposited at the Museu da Ciência da Universidade de Coimbra, Portugal (MCUC), Paratype 5 at the Zoologische Staatssammlung München, Germany (ZSM) and Paratype 8 at the Museum National d'Histoire Naturelle, Paris, France (MNHN).

**Faecal pellets, spawning and preparations:** Samples of faecal pellets of the Holotype and Paratypes 4, 6, 8, 9, 13, 17, 18, 19, 20, 21 and 22 are preserved in 70% EtOH neutralized with Borax. One spawning collected at sea with Paratypes 17 and 18 and several egg masses made in the laboratory aquarium, larvae and eggs of Paratypes 17, 18, 21 and 22 are also preserved. In addition, 92 SEM preparations are preserved of: radula, spicules, labial disc, eggs, larval shell, faecal pellets and spicules of *Polymastia boletiformis* (Lamarck, 1815) and 79 LM preparations: radula, spicules of the foot, dorsal and ventral mantle, tubercles and rhinophores, faecal pellets and spicules of the sponge *Polymastia boletiformis* (Lamarck, 1815). Also preserved are 383 preparations of histological sections of Paratype 4, 941 preparations of histological sections of Paratype 15, 81 preparations of histological sections of the genital system of Paratype 17 and 6 preparations of histological sections of the penis of Paratype 10. All this material has also been

deposited at the Museo de Historia Natural of the Universidade de Santiago de Compostela, Galicia, Spain (MHN-USC 10119).

## DESCRIPTION

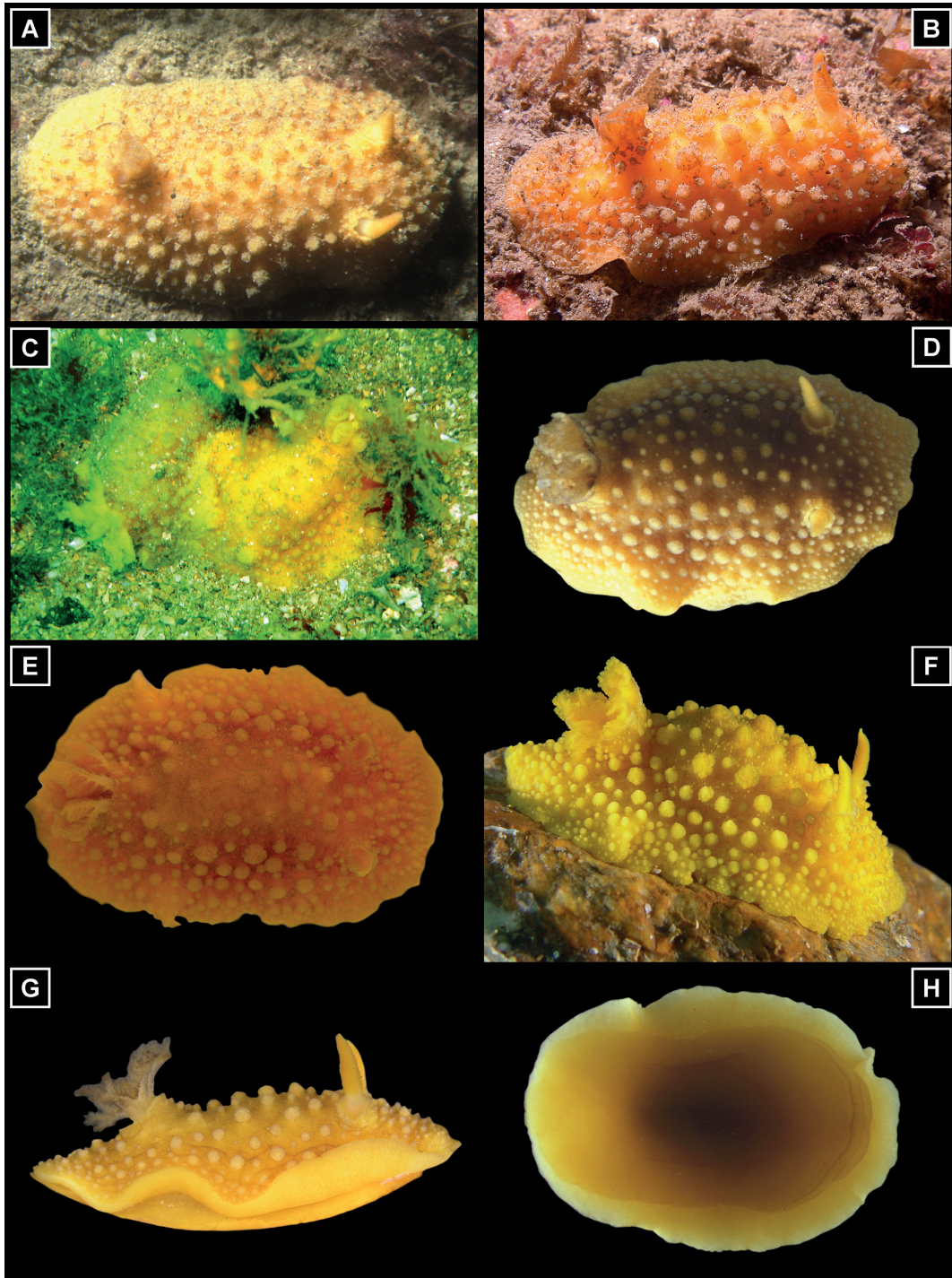
### External Anatomy

**Habitus:** The general colour of live animals varies from yellow to yellow-orange, although yellow specimens predominate (Fig. 2). The entire dorsal surface is finely dotted with black dots, which are only visible under the stereoscopic microscope. These dots increase their density in the mid-lateral areas of the notum, forming two somewhat darker bands running from the rhinophores to the gill (Figs. 2E,F). The outline of the mantle is oval to oblong, dorsally strongly convex, elevated, with a very hard consistency covering the whole animal, even when it is moving; the semicircular metapodium never protrudes (Figs. 2G,H). It possesses two small eyes, located between the two lobes of the blood gland, but they are not visible externally (Figs. 2A-G, 8B). The size of collected specimens varies between 32 and 76 mm in length and 18 and 55 mm in width, although the most frequent size fluctuates between 40-55 mm in length and 30-40 mm in width; the length/width ratio ranges from 1.3 to 1.8.

The entire dorsum is covered with rounded, slightly pointed, rough-surfaced tubercles with a small, broad basal stalk (Figs. 2A-G, 3G,H). In lateral view the tubercles have a conical appearance (Fig. 3H) and their colour is the same as that of the mantle with the same black stippling. The rough surface of the tubercles has an orange-peel appearance. The lattice of radial spicules giving shape to the tubercle can be seen through transparency, with the tips of the spicules directed towards the surface but not protruding outwards.

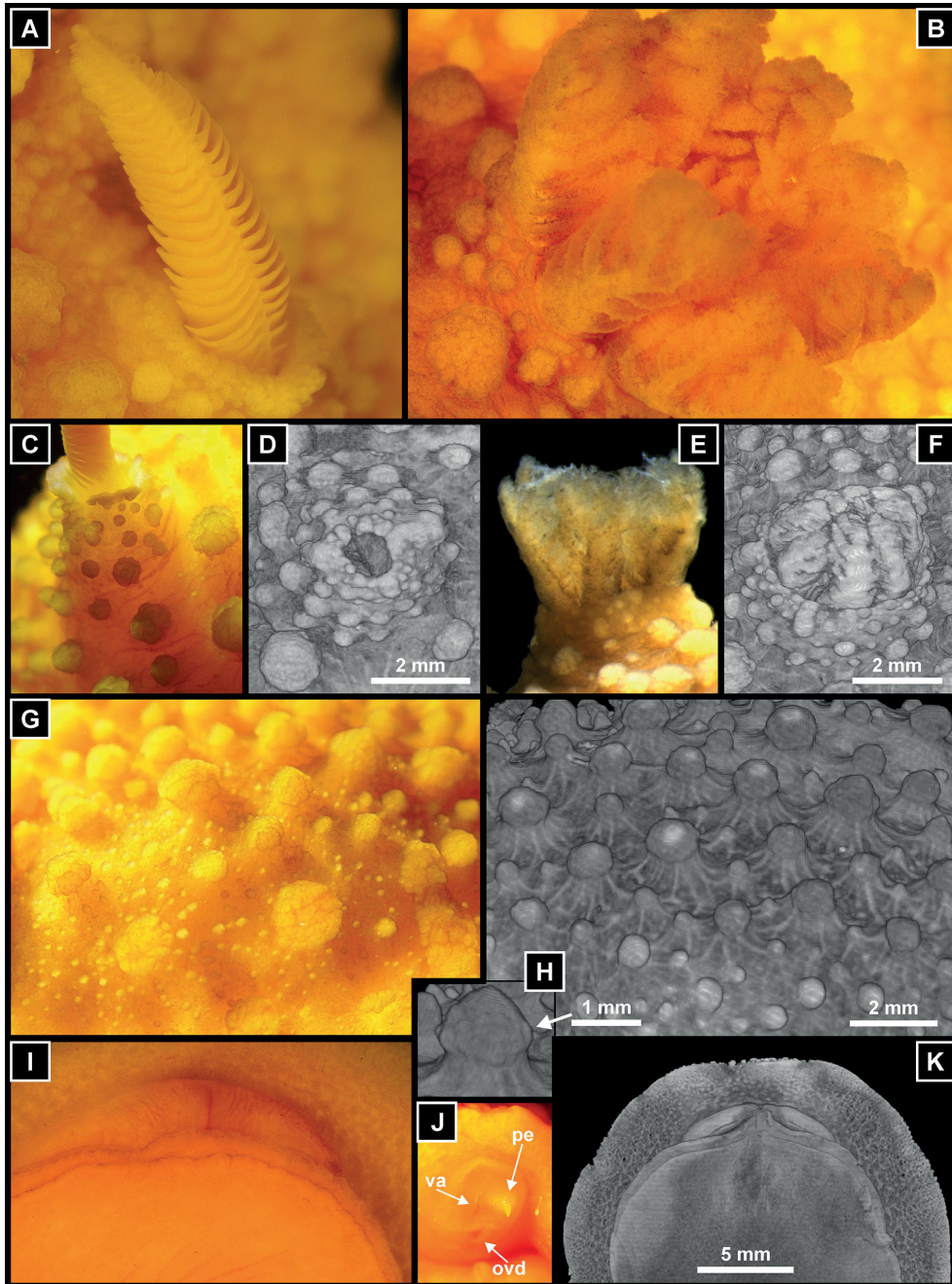
The tubercles range in size from large to very small, with various sizes in between so they cannot be grouped into any particular category, and with no consistent distribution. All sizes are present throughout the dorsum of the mantle, although the larger ones are much more abundant in the central part of





**Figure 2.** *Doris adrianae* sp. nov. *Habitus* and external appearance *in vivo*. **A:** Holotype 02020892, dorsal view in habitat, 65 mm. **B:** O Grelle 01250404, lateral view in habitat, 40 mm. **C:** Paratypes 17 and 18 01290806, top view of the two specimens copulating in habitat, 71-63 mm. **D:** Paratype 2, 01060786, dorsal view in aquarium, 40 mm. **E:** Paratype 19, 01220807, dorsal view in aquarium, 76 mm. **F:** Paratype 19, 01220807, lateral view in aquarium, 76 mm. **G:** Paratype 15, 03040106, lateral view in aquarium, 51 mm. **H:** Paratype 2, 01060786, ventral view in aquarium, 40 mm.

**Figura 2.** *Doris adrianae* sp. nov. *Habitus* y aspecto externo *in vivo*. **A:** Holotipo 02020892, vista dorsal en hábitat, 65 mm. **B:** O Grelle 01250404, vista lateral en hábitat, 40 mm. **C:** Paratipos 17 y 18 01290806, vista superior de dos ejemplares copulando en hábitat, 71-63 mm. **D:** Paratipo 2, 01060786, vista dorsal en acuario, 40 mm. **E:** Paratipo 19, 01220807, vista dorsal en acuario, 76 mm. **F:** Paratipo 19, 01220807, vista lateral en acuario, 76 mm. **G:** Paratipo 15, 03040106, vista lateral en acuario, 51 mm. **H:** Paratipo 2, 01060786, vista ventral en acuario, 40 mm.



**Figure 3.** *Doris adrianae* sp. nov. Details of the external appearance. **A:** Rhinophore *in vivo*, Paratype 19, 01220807-01, 76 mm. **B:** Branchial leaves, Paratype 19, 01220807-01, 76 mm. **C:** Rhinophoral sheath, Paratype 19, 01220807-01, 76 mm. **D:** Rhinophoral sheath, Paratype 16, 01240510, 41 mm. **E:** Branchial leaves and branchial sheath, Paratype 2, 01060786-02, 40 mm. **F:** Branchial sheath, Paratype 16, 01240510, 41 mm. **G:** Dorsal tubercles, Paratype 19, 01220807-01, 76 mm. **H:** Dorsal tubercles and detail of a tubercle, Paratype 6, 05050896, 40 mm. **I:** Oral lobes and anterior edge of bilaminated foot, Paratype 15, 03040106, 51 mm. **J:** External genital atrium, Paratype 19, 01220807-01, 76 mm. **K:** Ventral view of the anterior third of the animal, Paratype, 16 01240510, 41 mm. (A,B,C,E,G,I,J *in vivo*. D,F,H,K Micro-CT).

**Figura 3.** *Doris adrianae* sp. nov. Detalles del aspecto externo. **A:** Rinóforo *in vivo*, Paratipo 19, 01220807-01, 76 mm. **B:** Branquias, Paratipo 19, 01220807-01, 76 mm. **C:** Vaina rinofórica, Paratipo 19, 01220807-01, 76 mm. **D:** Vaina rinofórica, Paratipo 16, 01240510, 41 mm. **E:** Branquias y vaina branquial, Paratipo 2, 01060786-02, 40 mm. **F:** Vaina branquial, Paratipo 16, 01240510, 41 mm. **G:** Tubérculos dorsales, Paratipo 19, 01220807-01, 76 mm. **H:** Tubérculos dorsales y detalle de un tubérculo, Paratipo 6, 05050896, 40 mm. **I:** Lóbulos orales y borde anterior del pie bilaminado, Paratipo 15, 03040106, 51 mm. **J:** Atrio genital externo, Paratipo 19, 01220807-01, 76 mm. **K:** Vista ventral del tercio anterior del animal, Paratipo, 16 01240510, 41 mm. (A,B,C,E,G,I,J *in vivo*. D,F,H,K Micro-CT).

the notum and in the two darker lateral bands (black dots) from the rhinophores to the gills. In general, the size of the large tubercles decreases towards the edges of the mantle, where the smaller ones are much more abundant (Figs. 2A,B,D,F,G, 3G,H). Very small tubercles are scattered over the entire surface of the notum, including the rhinophoral sheaths and gill sheath, being slightly more abundant on the dorsal half and on the edge of the notum (Figs. 2C-H).

The rhinophores are elongate, narrow, slightly curved backwards with 29-34 lamellae in the upper two thirds of their length and with a slightly truncated conical basal stalk occupying the lower third (Figs. 2F,G, 3A). Yellow lamellae with slight black stippling, with the spicules arranged radially; they are arched and arranged alternately to right and left in a zigzagging frontal line. The tip of the rhinophore is a small truncated cylinder and the rhinophore stalk is white.

Gill with 6-8 tripinnate leaves, which appear to be bipinnate, as the tertiary branching is only visible at high magnification (Figs. 2A-G, 3B,E). In some specimens it has been observed that the two posterior leaves on each side each arise from a single basal bifurcation. The colour of the gill is quite variable, as in most specimens they are yellow to yellow-orange like the mantle (Figs. 2F, 3B), while in others they are whitish (Figs. 2G, 3E). All leaves show black dots like those on the dorsum, less dense on the rachis, but the rest of the leaf has different densities that give it a more or less dark colour, especially on the whitish leaves (Figs. 2G, 3B,E). The anus opens at the end of the anal papilla in the centre of the gills and is yellowish with few dots.

The rhinophoral and branchial sheaths are complete, raised, yellow with small to medium-sized tubercles on the edge (Figs. 3A-F). The two sheaths are of the same height, but the branchial sheath is much wider; the rhinophoral sheath may be as long as one-third the length of the rhinophore. When the rhinophores and gill retract, the sheaths close completely, bringing the edges closer together like a sphincter (Figs. 3C-F). The rhinophores are retracted by the action of three pairs of

small muscles inserted at the base of the stalk and diverging forwards, medially and posteriorly; the gill is also retracted by the action of three pairs of muscles, but of larger size than those of the rhinophores.

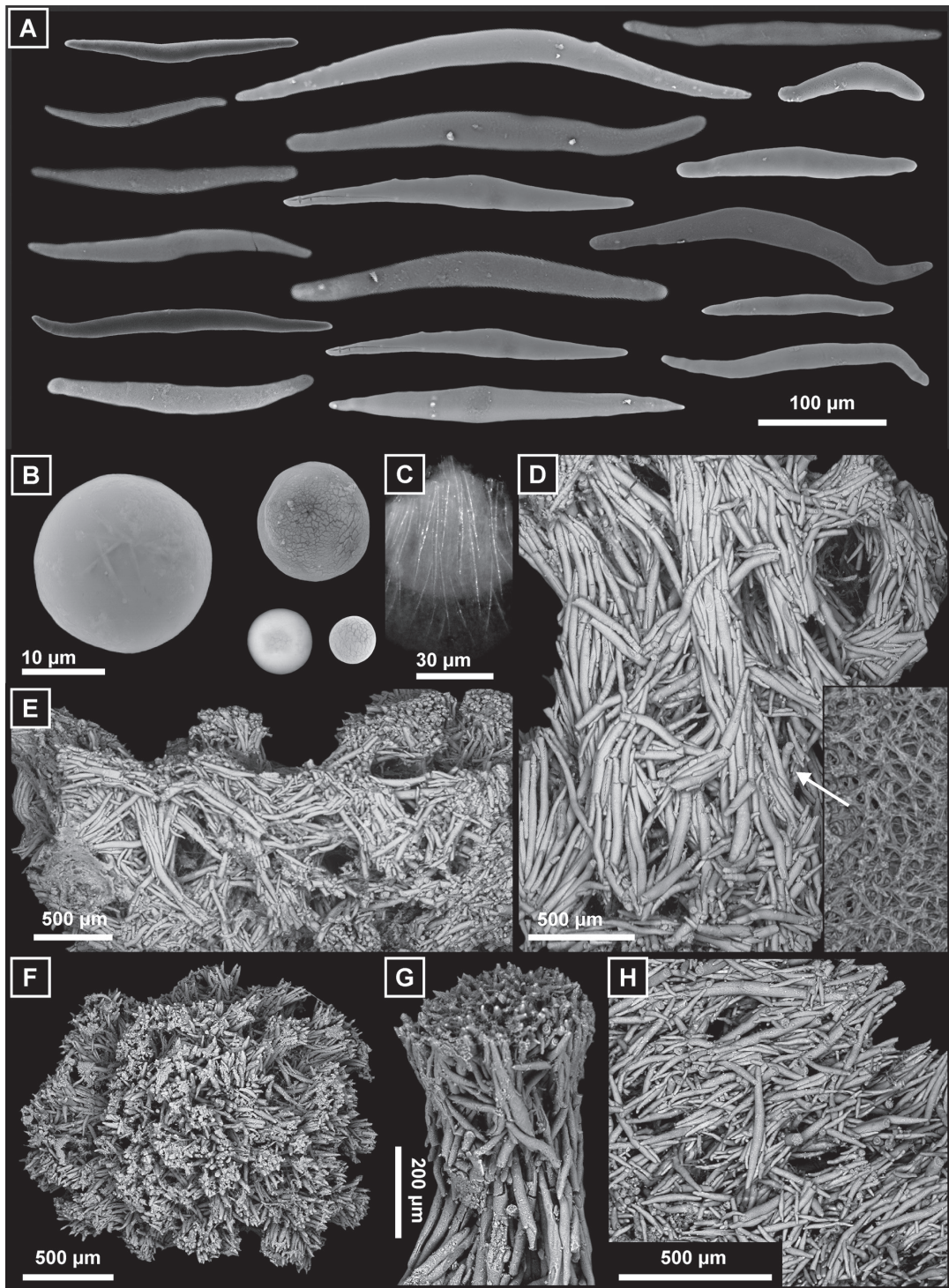
The mantle is ventrally slightly paler in colour than the dorsum, being the dense net of spicules visible through transparency (Figs. 2G-H). The foot, head and epipodium are more orange in colour than the rest of the animal, which is more yellow, with very few black dots. On the head, on the sides of the mouth, there are two flattened lobes without oral tentacles. The anterior edge of the foot is complete, without any notch and grooved on the anterior quarter, the upper lamella protruding more than the lower one (Figs. 2H, 3I,K). At rest and during movement the foot does not protrude from the edge of the mantle. However, very exceptionally, when the movement is very fast the posterior part of the metapodium protrudes slightly (Figs. 2G,H).

### Internal Anatomy

**Skeletal structure:** *Doris adrianae* sp. nov. has numerous calcareous spicules throughout its body, except in the visceral organs, which are arranged in a dense network, forming an armoured skeletal structure (Fig. 6A). This gives the animal a very compact consistency, clearly perceptible to the touch when specimens are collected.

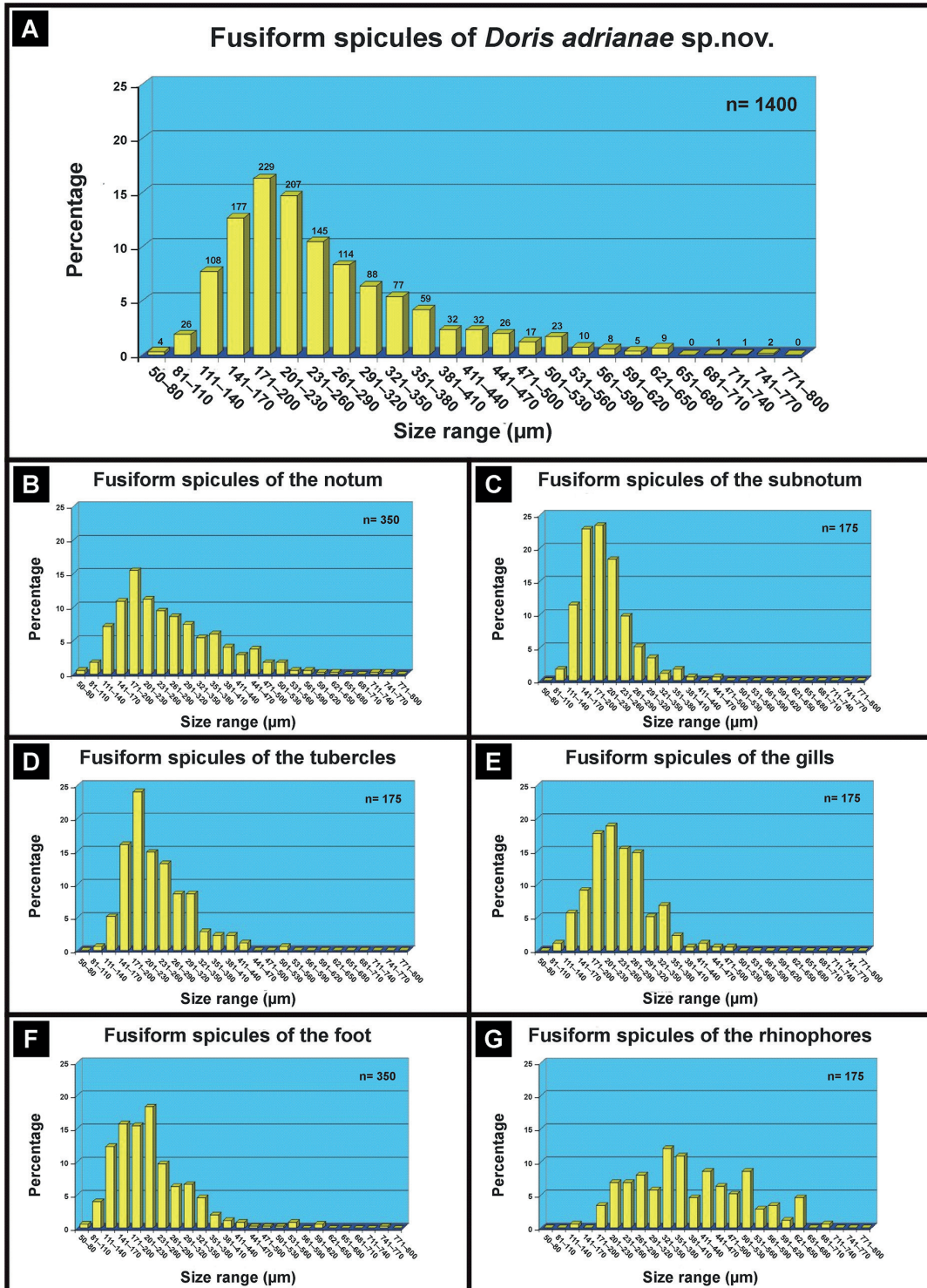
*Doris adrianae* sp. nov. has two types of spicules, some monoaxonic fusiform, irregular, elongated and with blunt ends (Fig. 4A), ranging in size from 70-770  $\mu\text{m}$  in length, with sizes between 111-320  $\mu\text{m}$  being more abundant (Fig. 5A). A second type of spicules are globose spherules, practically spherical, with a smooth surface and ranging in size from 5-15  $\mu\text{m}$  in diameter (Fig. 4B).

Fusiform spicules are more or less circular but irregular in section, with the thickest part located more or less in the centre of the spicule and with blunt ends, almost never acuminate. Although they are generally rectilinear, almost all show some degree of greater or lesser curvature, some being very curved and even with one of the ends bent at 45° (Fig. 4A). Their



**Figure 4.** *Doris adrianae* sp. nov. Skeletal structure. **A:** Different shapes and sizes of the fusiform spicules. **B:** Spherules. **C:** Spicules in a rhinophore lamina, Paratype 10 01080598-02, 45 mm. **D:** Skeletal structure of ventral mantle. **E:** Sagittal skeletal structure of lateral mantle. **F:** Radial spicular arrangement in a tubercle. **G:** Spicular arrangement of tubercle stalk. **H:** Spicular arrangement of foot. (D-H: Paratype 16 01240510, 41 mm). (A,B,D,E,F,G,H SEM. C LM).

**Figura 4.** *Doris adrianae* sp. nov. Estructura esquelética. **A:** Diferentes formas y tamaños de las espículas fusiformes. **B:** Esférulas. **C:** Espículas en una lámina del rinóforo, Paratipo 10 01080598-02, 45 mm. **D:** Estructura esquelética del manto ventral. **E:** Estructura esquelética sagital del manto lateral. **F:** Disposición espicular radial en un tubérculo. **G:** Disposición espicular del tallo del tubérculo. **H:** Disposición espicular del pie. (D-H: Paratipo 16 01240510, 41 mm). (A,B,D,E,F,G,H SEM. C MO).



**Figure 5.** *Doris adrianae* sp. nov. Size distribution of fusiform spicules. **A:** Lengths of total fusiform spicules (n = 1400). **B:** Lengths of spicules of notum (n = 350). **C:** Lengths of spicules of subnotum (n = 175). **D:** Lengths of spicules of tubercles (n = 175). **E:** Lengths of gill spicules (n = 175). **F:** Lengths of foot spicules (n = 350). **G:** Lengths of rhinophore spicules (n = 175).

**Figura 5.** *Doris adrianae* sp. nov. Distribución de tamaños de las espículas fusiformes. **A:** Longitudes de las espículas fusiformes totales (n = 1400). **B:** Longitudes de las espículas del notum (n = 350). **C:** Longitudes de las espículas del subnotum (n = 175). **D:** Longitudes de las espículas de los tubérculos (n = 175). **E:** Longitudes de las espículas de las branquias (n = 175). **F:** Longitudes de las espículas del pie (n = 350). **G:** Longitudes de las espículas del rinóforo (n = 175).

surface is smooth, without extensions or protuberances; however, some spicules, especially the larger ones, may have a series of small, irregularly distributed protuberances at their apices (Fig. 4A).

SEM microanalysis of the fusiform spicules showed that they are composed of: Mg, Ca, O, C and F, elements that crystallise amorphously, although when subjected to 400 °C they recrystallise in the form of calcite, brucite, fluorite and fluorapatite. SEM microanalysis of the spherules showed that they are composed of Ca, O and C.

Fusiform spicules are found on the mantle (notum and subnotum), tubercles, gills, foot and rhinophores. Their size distribution is more or less similar to all others, except for the rhinophore spicules which show a different size distribution (Figs. 5A-G). Globular-shaped spherules are found only on the mantle, rhinophores and at the base of the tubercles.

In the different body areas where these spicules are present, they are not arranged in the same way. For instance, in the mantle, the fusiform spicules form a network composed of a series of multispicular bundles that form a skeleton which is exclusively made up of agglutinated fusiform spicules, mostly arranged in the same direction. The crisscrossed arrangement of these bundles in the ventral mantle or subnotum delimits small spaces of different shapes, forming a triangular or polygonal reticulation (Figs. 4D, 6G). The spicules of the subnotum have a smaller size range, 86% of which are restricted to a range of 111-260  $\mu\text{m}$  in length (Fig. 5C).

In transversal section of the mantle (Fig. 6C) the multispicular ascending tracts branch and intertwine more densely at the mantle margins (Fig. 4E). In the dorsal mantle or notum where the tubercles are located, the ascending multispicular bundles show larger gaps between them, as they converge at the base of the tubercles. The greater the number and size of the converging tracts are, the larger the tubercle (Figs. 6C,F). This originates a thick spicular stem of the tubercle (Fig. 4G), from the upper end of the stem, a broad bundle of spicules is arranged radially with their ends

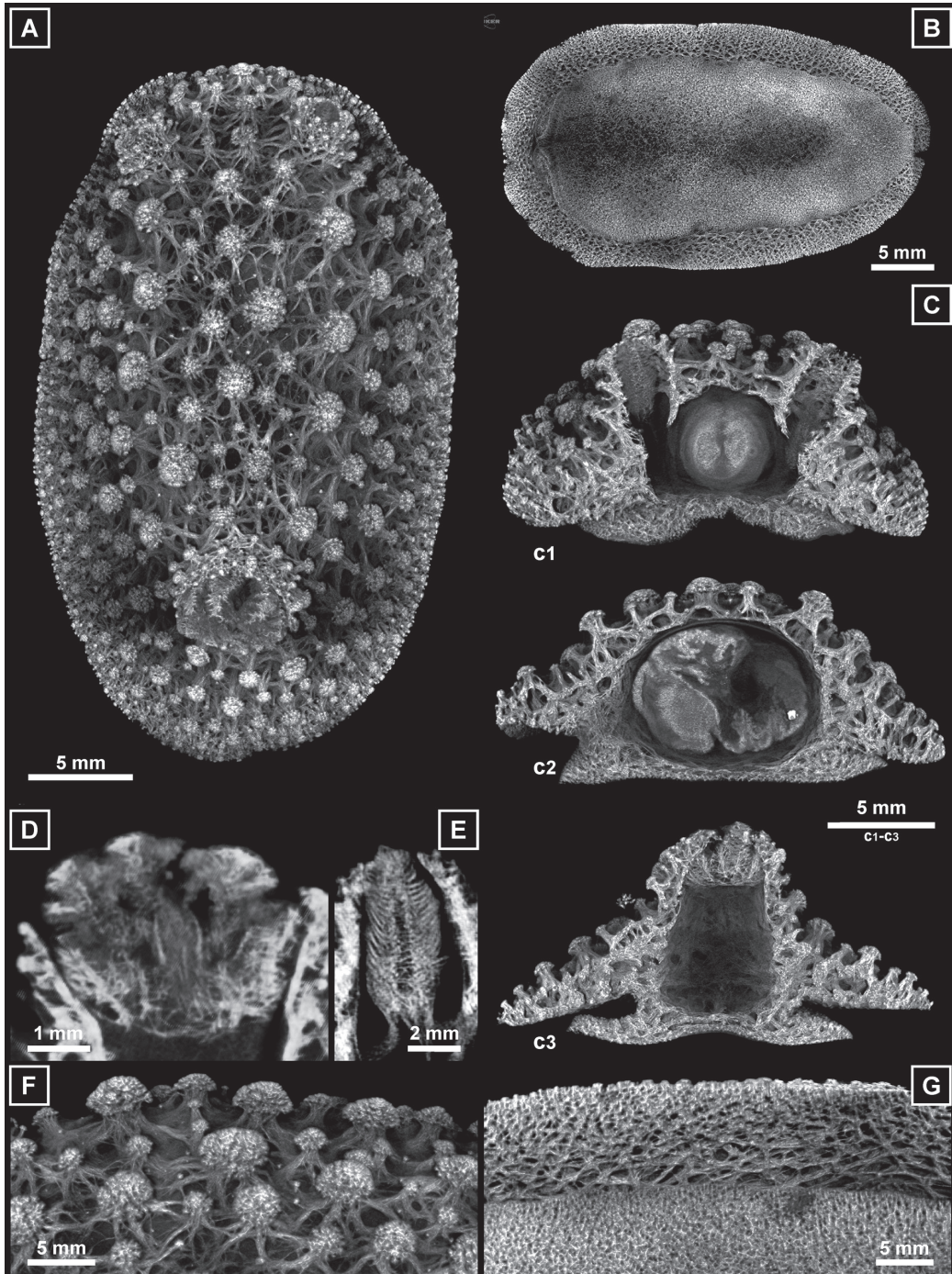
outwards but without crossing the epidermis, forming a hemispherical set (Figs. 4F, 6F). The mantle spherules are arranged in an apparently irregular shape and are distributed throughout the mantle up to the base of the tubercles where there are no spherules. The spicules of the notum have the largest size range between 70-770  $\mu\text{m}$  in length, of which 93% are restricted to a range of 111-470  $\mu\text{m}$  in length (Fig. 5B).

In the mantle tubercles there are only fusiform spicules that have a reduced size range, very similar to those of the foot, as 85% of the spicules are circumscribed to a range of 141-320  $\mu\text{m}$  in length (Fig. 5D). In the foot there are only fusiform spicules which form a less complex but denser net than in the mantle (Fig. 4h, 6G), because the spicules do not form multispicular bundles but an irregular and complex net of abundant spicules. The spicules of the foot have a smaller size range than the dorsal mantle, 93% of which are restricted to a range of 110-350  $\mu\text{m}$  in length (Fig. 5F).

Both types of spicules are present in the rhinophores, the fusiform spicules are irregularly arranged parallel to the axis of the rhinophore stalk while in the lamellae they are less numerous but arranged horizontally radially (Fig. 4C, 6E); the spherules in the rhinophore are only irregularly placed on the stalk, there are none in the lamellae. The rhinophore spicules show a different size distribution, as 99% of the spicules are distributed in a size range between 180-650  $\mu\text{m}$  in length, with no more than 2 spicules outside this range (Fig. 5G).

Finally, only fusiform spicules are found on the branchial leaves and they are irregularly placed, from the base to the middle of the rachis, being less numerous than in the other parts of the animal. In the gills, the size range is small, as 94% of the spicules are confined to a size range between 111-350  $\mu\text{m}$  in length (Fig. 5E).

As can be seen in figure 6 (Figs. 6A-G) *Doris adrianae* sp. nov. presents a very complex skeletal structure, constituting a very characteristic armature in the different body areas. In the images of this figure only the skeletal structure is shown, as the organic matter has



**Figure 6.** *Doris adrianae* sp. nov. Skeletal structure. **A:** Skeletal structure of the dorsal mantle and sheaths of the rhinophores and branchial leaves. **B:** Skeletal structure of ventral mantle and foot. **C:** Skeletal structure in cross-section: c1 at the level of the rhinophores, c2 at the level of the genital atrium and c3 at the level of the branchial leaves. **D:** Spicule density on branchial leaves. **E:** Density of spicules in the right rhinophore. **F:** Detail of the skeletal structure of the dorsal mantle. **G:** Detail of the skeletal structure of the ventral mantle and the edge of the foot. **A-G:** Paratype 16 01240510, 41 mm. (A-G Micro-CT).

**Figura 6.** *Doris adrianae* sp. nov. Estructura esquelética. **A:** Estructura esquelética del manto dorsal y vainas de rinóforos y branquias. **B:** Estructura esquelética del manto ventral y del pie. **C:** Estructura esquelética en sección transversal: c1 a nivel de los rinóforos, c2 a nivel de la atrio genital y c3 a nivel de las branquias. **D:** Densidad de espículas en las branquias. **E:** Densidad de espículas en el rinóforo derecho. **F:** Detalle de la estructura esquelética del manto dorsal. **G:** Detalle de la estructura esquelética del manto ventral y del borde del pie. **A-G:** Paratipo 16 01240510, 41 mm. (A-G Micro-CT).

been removed, except in the visceral cavity. Despite this complex structure, it neither limits nor hinders the animal's ability to move, its body shape or adaptation to the substrate surface (Figs. 2A-G).

**Digestive system:** The digestive system occupies a large part of the visceral cavity (Figs. 9A-D) and begins in a mouth flanked by two oral lobes (Figs. 3I,K). The oral mass is composed of a rounded buccal bulb and a shorter glandular oral tube. The posterior part of the oral tube has six strong retractor muscles (Fig. 7K), three on each side, which attach to the body wall at the posterior part of the buccal bulb. The intermediate pair of muscles bifurcates almost at the beginning into two divergent muscles (mot2 in Fig. 7K). The buccal bulb is rounded in shape, almost twice as large as the oral tube, with a radular sac emerging from the posterior ventral surface and recurved posteriorly (Fig. 7K). From the posterior-lower half of the buccal bulb arise a pair of long, strong retractor muscles, one on each side, which insert into the body wall, roughly towards the middle of the visceral cavity where the hermaphrodite and digestive glands begin (Fig. 7K).

At the anterior end of the pharynx, towards the oral tube, there is a chitinous labial cuticle of pelted shape, the labial disc (Fig. 7G), whose frontal surface is smoothly roughened, while the surface of the central canal is much rougher (Fig. 7G). In the posterior part of the pharynx, the oesophagus opens at the upper end (Figs. 7I,K). Two long salivary glands connect with the pharynx on either side of the oesophageal junction, between the visceral loop and the oesophagus, on either side of the buccal ganglia (Figs. 7H-K); they are ribbon-shaped, wider in their initial section, and extend ventrally, running under the visceral mass to the mid-height of the stomach.

It has a multidenticulate radula (Fig. 7B), whose radular formula is 38-50 x 3-4;33-53;0;33-53;3-4. The radula lacks a rachidial tooth. The lateral teeth are of simple hooked shape, with a strong ridge more marked the larger the tooth, the tip of the tooth is slightly blunt and its surface is smooth (Figs.

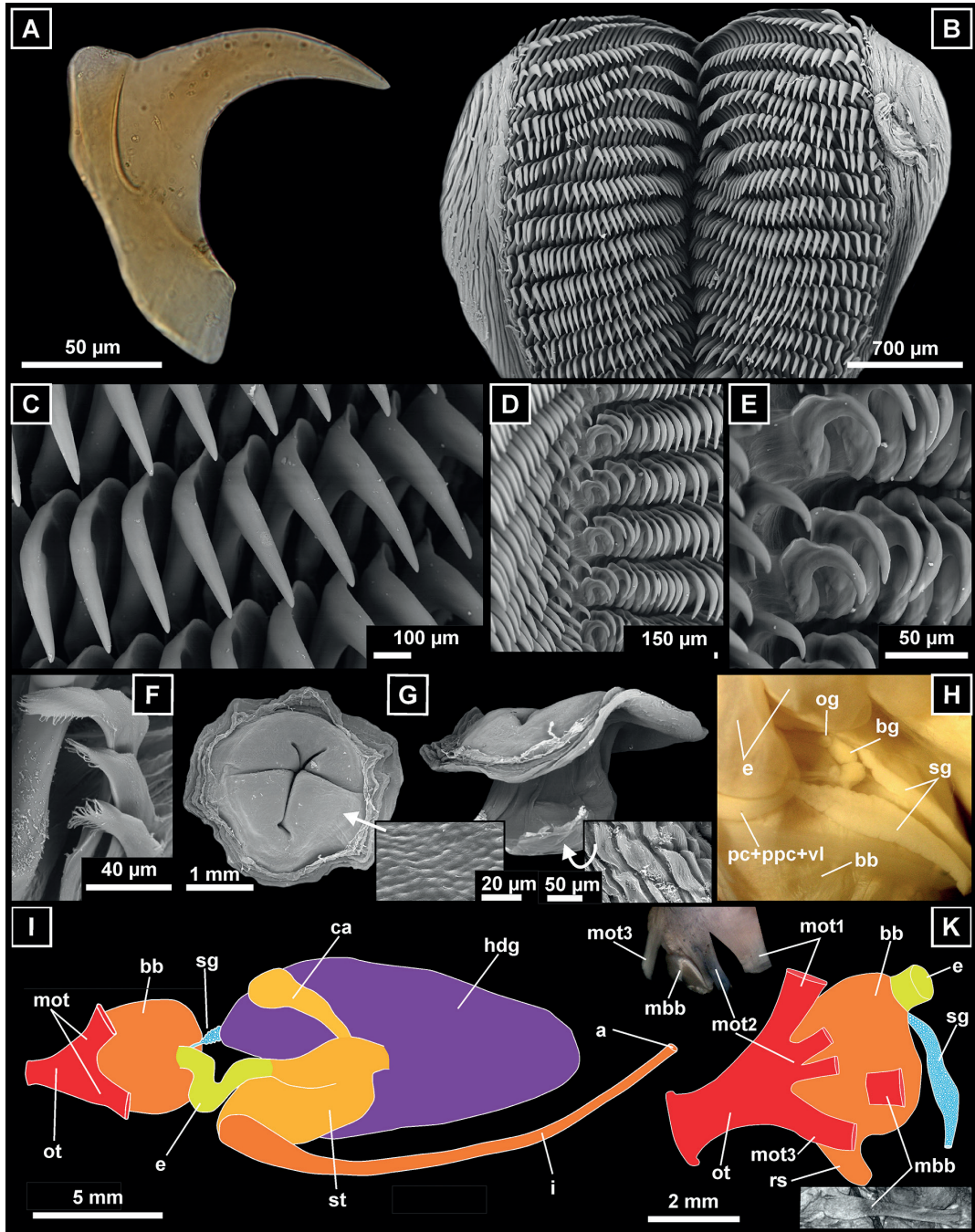
7A,C). The first central lateral teeth are more curved, being more hooked than the others (Figs. 7D,E). The marginal teeth are similar to the lateral ones but the hooked edge is not smooth but feathery, the first ones having a slightly hooked shape, while the outermost ones acquire a feathery spade-like appearance (Fig. 7F).

The oesophagus is long and curved and opens laterally to the stomach which is large, curved and of variable diameter, opening posteriorly to the digestive gland. There is a gastric caecum that opens into the stomach very close to where it connects to the oesophagus and digestive gland. From the left anterior part of the stomach a long intestine emerges dorsally, it crosses across the visceral mass dorsally and runs along the right side of the animal, until it enters laterally into the gill crown, in the centre of which the anus is located (Figs. 7I, 9C,D). The digestive gland is interspersed with the hermaphrodite gland, although most of the gonad surrounds the digestive gland superficially (Fig. 8G).

**Nervous system:** In the central nervous system (Fig. 8A) the cerebral and pleural ganglia are fused and distinct from the pedal ganglia. There are four cerebral nerves emerging from each cerebral ganglion and three pleural nerves emerging from each pleural ganglion. From each of the pedal ganglia there are 5 pedal nerves. The rhinophore nerves emerge one from each cerebral ganglion, from a position more centred in the ganglion. The buccal ganglia (Figs. 7H, 8A) are located posterior to the buccal bulb, below the beginning of the oesophagus and are connected to the cerebral ganglia by two narrow, relatively short nerves. Very close to the buccal ganglia are the gastro-oesophageal ganglia. The optic ganglion is close to the insertion of the rhinophore nerve. The pedal and the parapedal commissures are together in the visceral loop.

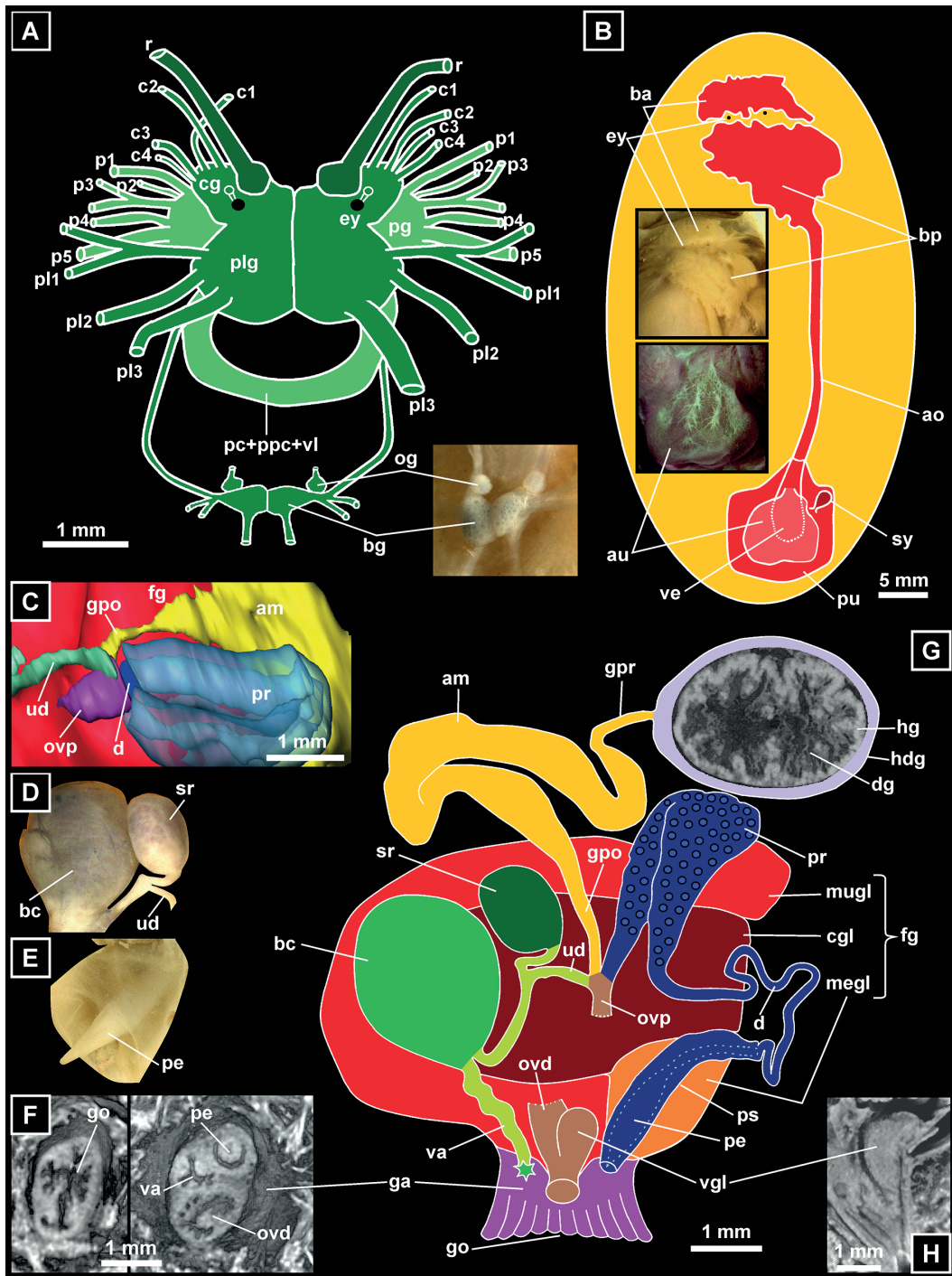
**Circulatory system:** The circulatory system consists of a heart situated in the posterior part of the animal. The pericardial cavity is more or less circular, anterior to the branchial leaves ring and dorsal to the hermaphrodite and digestive glands. The auricle is funnel-shaped





**Figure 7.** *Doris adrianae* sp. nov. Digestive system, radula and labial cuticle. **A:** Radula tooth, Paratype 10, 01080598-02, 45 mm. **B-C:** Complete radula and detail of the mid-lateral teeth of the radula, Paratype 17, 01220807-01, 71 mm. **D-E:** Inner lateral teeth hooked, Paratype 7, 05030597, 60 mm. **F:** Outer marginal teeth plumose, Paratype 17, 01220807, 71 mm. **G:** Labial cuticle, Paratype 1, 01060786-01, 50 mm. **H:** Salivary glands, buccal ganglia and gastro-oesophageal ganglia, Paratype 7, 05130597-01, 60 mm. **I:** Schematic drawing of the regionalization of the digestive system. **K:** Schematic drawing of oral tube and buccal bulb. (A,H LM. B-G SEM).

**Figura 7.** *Doris adrianae* sp. nov. Aparato digestivo, rádula y cutícula labial. **A:** Diente de la rádula, Paratipo 10, 01080598-02, 45 mm. **B-C:** Rádula completa y detalle de los dientes laterales medios de la rádula, Paratipo 17, 01220807-01, 71 mm. **D-E:** Dientes laterales internos ganchudos, Paratipo 7, 05030597, 60 mm. **F:** Dientes marginales externos plumosos, Paratipo 17, 01220807, 71 mm. **G:** Cutícula labial, Paratipo 1, 01060786-01, 50 mm. **H:** Glándulas salivales, ganglios bucales y ganglios gastroesofágicos, Paratipo 7, 05130597-01, 60 mm. **I:** Dibujo esquemático de la regionalización del aparato digestivo. **K:** Dibujo esquemático del tubo oral y del bulbo bucal. (A,H OM. B-G SEM).



**Figure 8.** *Doris adrianae* sp. nov. Internal anatomy. **A:** Central nervous system, Paratype 7, 05130597-01, 60 mm. **B:** Circulatory system, Paratype 7, 05130597-01, 60 mm. **C:** Confluent ducts in the distal oviduct. **D:** Bursa copulatrix and seminal receptacle, Paratype 7, 05130597-01, 60 mm. **E:** Penis, Paratype 17, 01220807-01, 71 mm. **F:** Openings in the genital atrium, Paratype 16 01240510, 41 mm. **G:** Schematic drawing of the regionalization of the reproductive system. **H:** Vestibular gland, Paratype 16 01240510, 41 mm. (A,B,D,E LM. C 3D reconstruction with AVIZO 6.4. F,H Micro-CT). **Figura 8.** *Doris adrianae* sp. nov. Anatomía interna. **A:** Sistema nervioso central, Paratipo 7, 05130597-01, 60 mm. **B:** Aparato circulatorio, Paratipo 7, 05130597-01, 60 mm. **C:** Conductos confluentes en el oviducto distal. **D:** Bursa copulatrix y receptáculo seminal, Paratipo 7, 05130597-01, 60 mm. **E:** Pene, Paratipo 17, 01220807-01, 71 mm. **F:** Aberturas en el atrio genital, Paratipo 16 01240510, 41 mm. **G:** Dibujo esquemático de la regionalización del aparato reproductor. **H:** Glándula vestibular, Paratipo 16 01240510, 41 mm. (A,B,D,E OM. C Reconstrucción 3D con AVIZO 6.4. F,H Micro-CT).

and provided with numerous small vessels (Fig. 8B); ventricle below the auricle, elongated in shape. In a 32 mm specimen, 68 beats per minute were counted in the heart. From the heart, a dorsal vessel runs through the animal towards the anterior region, where a large blood gland is located above the central nervous system; the blood gland consists of two parts, an anterior and a posterior part, which is the largest (Fig. 8B), revealing the eyes between them. The renal syrinx is elliptical and located on the right dorsal side of the pericardium, near the base of the auricle (Fig. 8B).

**Reproductive system:** The reproductive system is triaulic and is located in a right latero-dorsal position between the buccal bulb and the hermaphrodite and digestive glands (Fig. 9B). The genital opening is located in the subnotum (Fig. 3J), close to the epipodium, in the anterior third of the animal's length, through which the genital atrium is accessed, into which the penis, vagina and distal oviduct open (Fig. 8F). The hermaphrodite gland is interspersed with the digestive gland which it wraps superficially (Fig. 8G). The preampullar gonoduct is short and narrow and joins the ampulla above the stomach and below the intestine. The ampulla is long, tubular and thick, and folds in on itself before narrowing into the postampullar gonoduct which is short and narrow connecting with the proximal oviduct (Figs. 8C,G, 9E,F,G). The penis is small, elongated, fusiform and unarmed (Fig. 8E), covered by a muscular penial sheath which has some fusiform spicules in its distal part. A narrow, long and sinuous deferent duct, emerges from the penis and opens into a large, tubular, granular prostate that folds back on itself, finally connecting through a short deferent duct to the proximal oviduct (Figs. 8C,G, 9E,F,G).

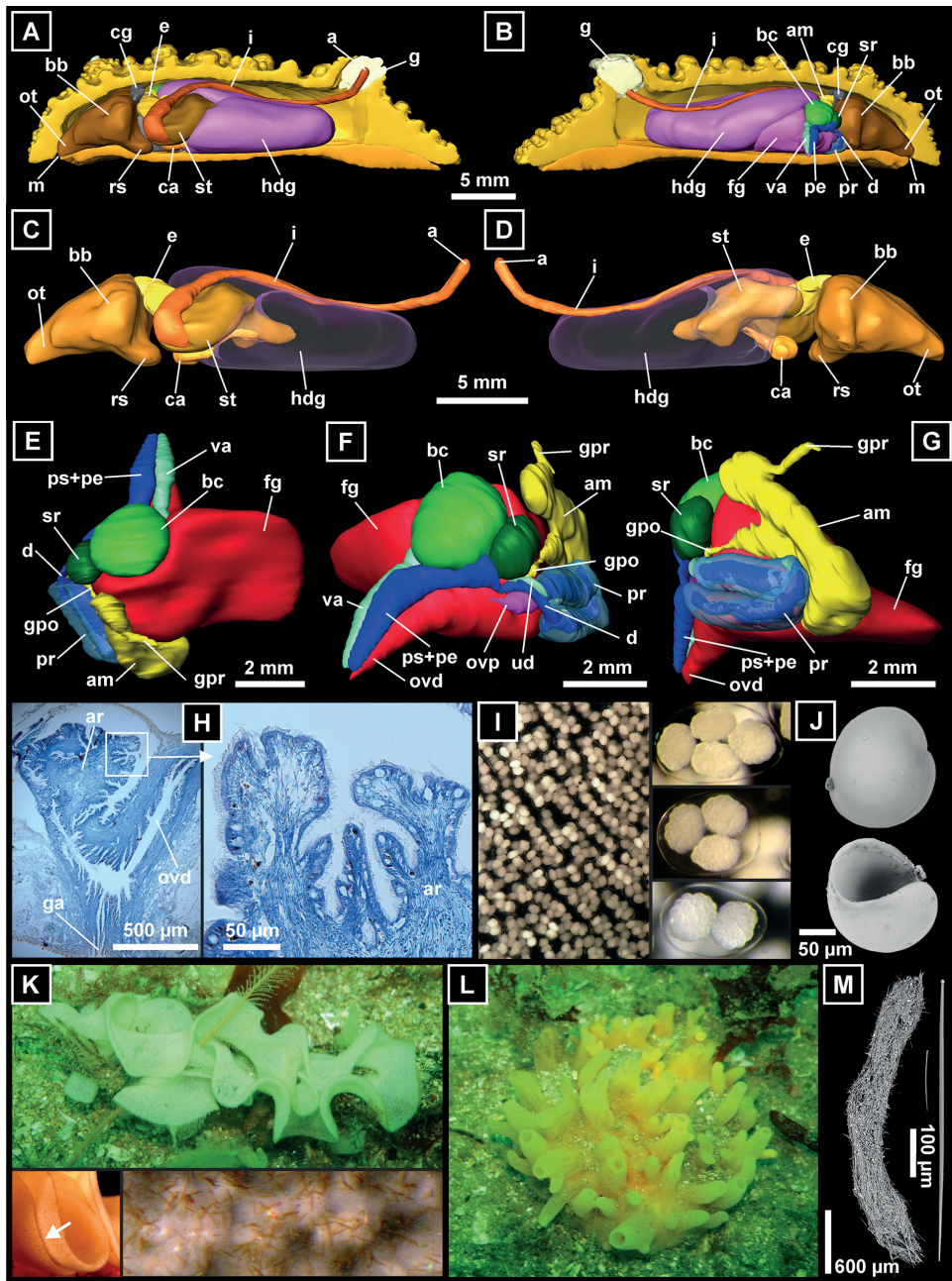
The vagina is short and wide and the proximal end of the vaginal duct joins the bursa copulatrix. From the bursa copulatrix another duct leads to the uterine duct and the seminal receptacle (Figs. 8C,D,G, 9E,F,G). The bursa copulatrix is large, globose and oval in shape, about three times larger than the seminal

receptacle which is also globose and oval. Both have smooth surfaces with slight bulges (Fig. 8D). The short uterine duct together with the deferent duct and the postampullary gonoduct open into the proximal oviduct which after a short section penetrates the female gland through the capsule gland (Fig. 8G).

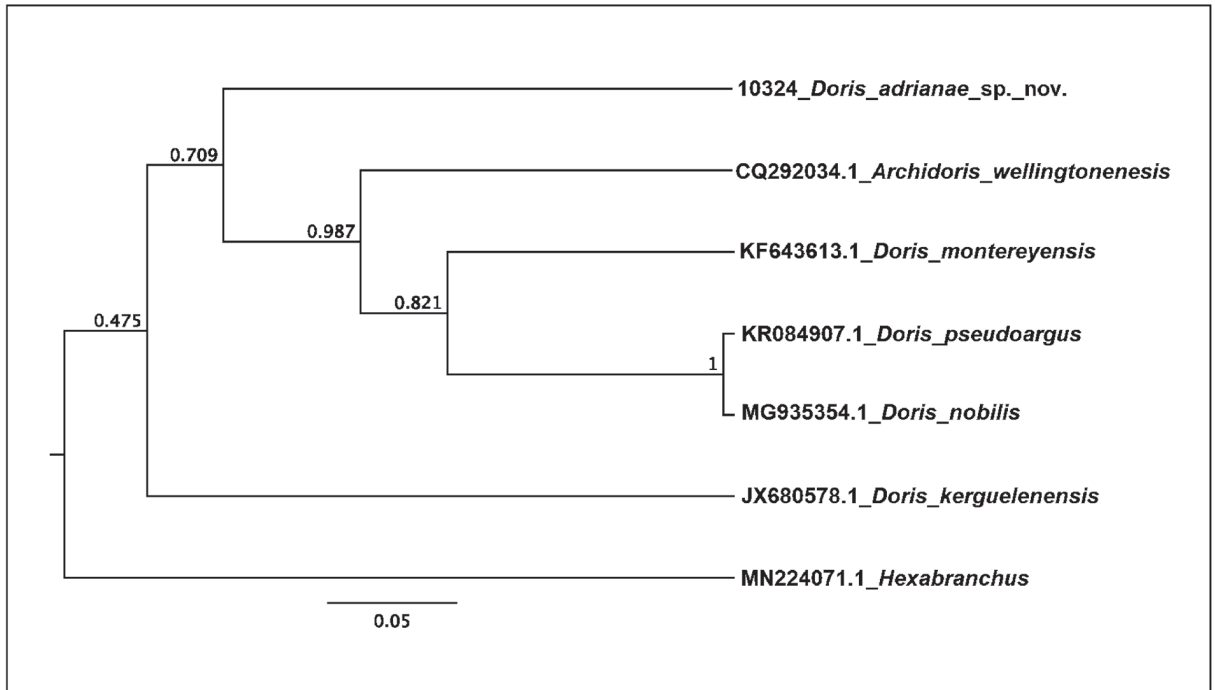
The capsule gland is centrally located and of medium size; the membrane gland, the smallest, is located in the ventral anterior area; the mucus gland, the largest, surrounds the others except in the anterior region (Fig. 8G). In the final section of the distal oviduct, near its opening in the genital atrium, there is a globular structure of glandular appearance which could be a vestibular gland, most likely as an adhesive region (Figs. 8G,H, 9H).

**DNA sequence data and phylogenetic analysis:** The *cox1-5'* sequence for Paratype 20, DNA is available under GenBank accession number MW602531. The genetic distance between *Doris adrianae* sp. nov. and other *Doris* species was large (mean proportion of identical sites =  $80.9\% \pm 1.5$  (S.D.)) although similar to the ones among *Doris* sequences available in GenBank (see Table 1). The bayesian phylogenetic tree shows *Doris adrianae* sp. nov. as sister species of a clade with *Doris wellingtonensis*, *Doris montereyensis*, *Doris pseudoargus* and *Doris nobilis*, with a relatively high support for the node (Bayesian posterior probability=0.709, Fig. 10).

**Spawning:** The spawning is hyaline white, slightly greyish and with the upper edge of the band brown, while the lower edge is hyaline. The spawning is in the form of a festoon ribbon with 2 to 2.5 turns and 26 to 31 waves (Fig. 9K). The height of the ribbon is 15 mm. The egg capsules have a linear arrangement from the upper to the lower edge, but oblique or arched across the width of the ribbon, although this tendency is more evident towards the upper edge and less noticeable in the lower half (Fig. 9I). The capsules have 1, 2, 3 or 4 eggs, but mostly two (Fig. 9I). On the upper edge of the ribbon the egg capsules are arranged almost close to the margin, while on the lower edge there is a small hyaline strip devoid of eggs which is the line of attachment



**Figure 9.** *Doris adrianae* sp. nov. 3D reconstruction of internal anatomy, adhesive region, feeding and spawning. **A-B:** Organs *in situ*, left (A) and right (B) sides. **C-D:** Digestive system in lateral view, left (C) and right (D). **E-G:** Reproductive system in dorsal (E), right anterior-lateral (F) and right posterior-lateral views (G). **H:** Adhesive region. **I:** Arrangement of eggs in the spawn and egg capsules *in vivo*. **J:** Larval shell (protoconch type I). **K:** Spawning in type locality (Castelo de San Felipe), brown upper edge (arrow) and fusiform spicules of the brown edge. **L:** The sponge *Polymastia boletiformis* (Lamarck, 1815) in type locality (Punta Fornelos). **M:** Faecal pellet full of spicules of *P. boletiformis* and small (133 μm) and large (586 μm) tylostyles of the faecal pellet. (A-G 3D reconstruction with AVIZO 6.4. H LM. J,M SEM. I,K,L *in vivo*). **Figura 9.** *Doris adrianae* sp. nov. Reconstrucción en 3D de la anatomía interna, región adhesiva, alimentación y desove. **A-B:** Órganos *in situ*, lateral izquierdo (A) y derecho (A). **C-D:** Aparato digestivo en vista lateral, izquierda (C) y derecha (D). **E-G:** Aparato reproductor en vista dorsal (E), antero-lateral derecha (F) y postero-lateral derecha (G). **H:** Región adhesiva. **I:** Disposición de los huevos en la puesta y cápsulas con huevos *in vivo*. **J:** Concha larvaria (protoconcha tipo I). **K:** Puesta en la localidad tipo (Castelo de San Felipe), borde superior marrón (flecha) y espículas fusiformes del borde marrón. **L:** La esponja *Polymastia boletiformis* (Lamarck, 1815) en la localidad tipo (Punta Fornelos). **M:** Pelota fecal repleta de espículas de *P. boletiformis* y tilostilos pequeños (133 μm) y grandes (586 μm) de las pelotas fecales. (A-G Reconstrucción 3D con AVIZO 6.4. H OM. J,M SEM. I,K,L *in vivo*).



**Figure 10.** *Doris adrianae* sp. nov. Phylogenetic tree resulting from BEAST analysis of *Doris adrianae* sp. nov. and *cox1-5'* sequences of *Doris* sequences downloaded from GenBank One GenBank sequence has been selected for each species. Node values represent Bayesian posterior probabilities.

**Figura 10.** *Doris adrianae* sp. nov. Árbol filogenético resultante del análisis BEAST de *Doris adrianae* sp. nov. y de las secuencias *cox1-5'* de *Doris* descargadas de GenBank Se ha seleccionado una secuencia de GenBank para cada especie. Los valores de los nodos representan las probabilidades posteriores bayesianas.

to the substratum. A preparation under light microscopic of the upper edge of the ribbon revealed that the brown colour of the edge was due to the presence of fusiform spicules like those found in adult specimens on the mantle and foot (Fig. 9K). The larval shell is a smooth type I protoconch (THOMPSON, 1961) with a large turn of the spire and a wide oval opening with a smooth edge (Fig. 9J).

**Habitat and associated fauna:** The three localities of the Ría de Ferrol where the specimens were collected between 11 and 20 m deep, have a very similar habitat, characterised by the existence of extensive, flat and low rocky outcrops of granodiorites, where a forest of the gorgonian *Leptogorgia lusitanica* (Stiasny, 1937) is located. By its proximity to a sandy-muddy sedimentary bottom, the rock surface is covered with a thin layer of fine sediment. The community beneath the gorgonian forest is devoid of large algae, but shows a wide diversity of sessile invertebrate species where certain species of sponges

*Polymastia boletiformis* (Lamarck, 1815), *Mycale (Aegogropila) antiae* Urgorri & Díaz-Agras, 2019, *Cliona celata* Grant, 1826, *Adreus fascicularis* (Bowerbank, 1866), *Desmacidon fruticosum* (Montagu, 1814), *Ciocalypta penicillus* Bowerbank, 1862 and *Haliclona (Haliclona) oculata* (Linnaeus, 1759), the cnidarians *Dynamena pumila* (Linnaeus, 1758), *Sertularella gayi* (Lamouroux, 1821), *Aglaophenia acacia* Allman, 1883, *Actinothoe sphyrodeta* (Gosse, 1858), *Epizoanthus arenaeus* (Delle Chiaje, 1823), *Caryophyllia smithii* Stokes & Broderip, 1828, *Eunicella verrucosa* (Pallas, 1766) and *Alcyonium digitatum* Linnaeus, 1758, the bivalve *Mimachlamys varia* (Linnaeus, 1758), the polychaete *Sabellaria alcocki* Gravier, 1906 and the ascidian *Stolonica socialis* Hartmeyer, 1903 and also other species of vagile invertebrates, such as the molluscs *Calliostoma zizyphinum* (Linnaeus, 1758), *Turritella communis* Risso, 1826, *Tritia reticulata* (Linnaeus, 1758), *Trapania tartanella* (Ihering, 1886 in IHERING, 1886b) and *Cadlina*

*laevis* (Linnaeus, 1767) and the echinoderms *Marthasterias glacialis* (Linnaeus, 1758), *Echinus esculentus* Linnaeus, 1758 and *Holothuria forskali* Delle Chiaje, 1823.

**Feeding behaviour:** The study of the contents of the faecal pellets obtained from live animals of *Doris adrianae* sp. nov. (Fig. 9M), as well as the digestive contents of the 4 dissected animals and that of the serial sections of two specimens (paratypes 4 and 15), revealed a spicular composition of tylostyles, identical to that of the sponge *Polymastia boletiformis* (Lamarck, 1815). This sponge has two types of tylostyles, some large (500-700 µm) fusiform, rectilinear, with a tylo at one end and the other ending in a point, and others small (125-190 µm) fusiform, slightly curved and with a scarcely marked tylo (Fig. 9M). The exclusive presence of *Polymastia boletiformis* (Lamarck, 1815) spicules in all the samples studied, without having found any other type of spicule, makes it possible to ensure that *Doris adrianae* sp. nov. feeds exclusively on the sponge *Polymastia boletiformis* (Lamarck, 1815), which is very abundant in the three type localities of the Ría de Ferrol (Fig. 9L) and that both species coincide remarkably in their colouring.

## DISCUSSION

According to the diagnosis of the genus *Doris* Linnaeus, 1758 established by VALDÉS (2002), *Doris adrianae* sp. nov. clearly fits within the genus *Doris* on the basis of the absence of a notch on the anterior margin of the foot, the labial cuticle lacking rodlets, a radula composed of simple, hamate teeth, a penis and vagina devoid of hooks and also on the fact that it matches the other diagnostic characters of the genus (VALDÉS, 2002).

Furthermore, according to some diagnostic characters of the other genera that comprise the family Dorididae Rafinesque, 1815, it could not be classified in any of them, as there are clear differences with *Doris adrianae* sp. nov. For example, the genus *Aphelodoris* Bergh, 1879 differs in that it does not have integumentary spicules and has a smooth dorsum without tubercles (VALDÉS, 2002).

*Doris adrianae* sp. nov. could not be classified within the genus *Conualevia* Collier & Farmer, 1964 because this genus has mantle glands and rhinophores almost smooth, with several irregular and inconspicuous lamellae (VALDÉS, 2002). The genus *Goslineria* Valdés, 2001 has several large sacs in the genital atrium, each containing a long, simple, flexible spine (VALDÉS, 2001), which are absent in *D. adrianae* sp. nov. It also does not belong in the genus *Artachaea* Bergh, 1881 in BERGH, 1881b, because *Artachaea* has oral tentacles and the penis is armed with spines (VALDÉS, 2002), which *Doris adrianae* sp. nov. lacks (VALDÉS, 2002). The genus *Pharadoris* Valdés, 2001, has a genital atrium with two large glands, each containing a long, bifid, rigid spine, which *Doris adrianae* sp. nov. does not have. The genus *Doriopsis* Pease, 1860 is characterised by a wide semicircular crown of gill leaves very different from those of *Doris adrianae* sp. nov.

Undoubtedly, the most reliable diagnostic character of *Doris adrianae* sp. nov. that clearly differentiates it from the rest of the species of the genus *Doris*, is the skeletal structure that forms a dense net of spicules, in a complex network of multispicular bundles.

Dorids are known to have tegumentary spicules, although most papers describe their presence as spicules radiating from the base of the tubercles and other vertical spicules supporting the tubercles (KRESS, 1981; CATTANEO-VIETTI, *et al.* 1993; BRODIE, 2004; EHRLICH, 2019; ORTEA & ESPINOSA, 2017; among others). Some authors make more detailed descriptions of the skeletal structure of dorids (GARCÍA, *et al.* 1986; PENNEY, 2006), in some use micro-computed tomography (ALBA-TERCEDOR & SANCHEZ-TOCINO, 2011; PENNEY, *et al.* 2018; PENNEY, *et al.* 2020) and include species of the genus *Doris* (PENNEY, 2008) such as *Doris montereyensis* Cooper, 1863, but none of these studies describe a skeletal structure as complex as that of *Doris adrianae* sp. nov. This complex skeletal structure could initially be interpreted as having a primarily defensive or at least deterrent function for potential predators (THOMPSON, 1960; ROS, 1976), but, probably in *D. adrianae* sp. nov. they also

have a structural function, a function that was described by CATTANEO-VIETTI, *et al.* (1993) in *Peltodoris atromaculata* Bergh, 1880, stating that they have an important role in determining the firmness, structure and architecture of the notum.

Of the genus *Doris* Linnaeus, 1758, 51 species are currently recognised as valid (WoRMS, 2021), generally with an external appearance different from that of *Doris adrianae* sp. nov. Of these, 13 are distributed along the Northeastern Atlantic which ranges from Sweden to Cape Verde. All these species, in addition to differing in skeletal structure, they exhibit other characters different from *Doris adrianae* sp. nov. For example, *Doris atypica* (Eliot, 1906), *Doris bicolor* (Bergh, 1884 in BERGH, 1884a), *Doris ocelligera* (Bergh, 1881 in BERGH, 1881a) and *Doris verrucosa* Linnaeus, 1758, have unipinnate branchial leaves and *Doris marmorata* Risso, 1818 and *Doris pseudoverrucosa* (Ihering, 1886 in IHERING, 1886a) show bipinnate branching and in addition these six species have rhinophoral sheaths with two lateral tubercles that close the rhinophoral cavity like two valves (ELIOT, 1906; ORTEA & MORO, 2017; BERGH, 1884a; PRUVOT-FOL, 1954; ORTEA, *et al.* 2014; CATTANEO-VIETTI, *et al.* 1990; VALDÉS, 2002; LIMA & SIMONE, 2015; IHERING, 1886a; SCHMEKEL, 1968; SCHMEKEL & PORTMANN, 1982). *Doris hayeki* Ortea, 1998 and *Doris morenoi* Ortea, 1989 also have unipinnate leaves, both exhibit hooked marginal teeth without denticles and their bursa copulatrix is slightly larger (x1.5) than the seminal receptacle (ORTEA, 1989, 1998). The remaining five species have tripinnate or bipinnate leaves, but *Doris nobilis* Odhner, 1907, *Doris sticta* (Iredale & O'Donoghue, 1923) and *Doris bertheloti* (d'Orbigny, 1839) have rhinophoral sheaths with two lateral tubercles and the marginal teeth are hooked without denticles (ODHNER, 1907, 1926; THOMPSON & BROWN, 1984; HUNNAM & BROWN, 1975; GAVAIA, *et al.* 2003; D'ORBIGNY, 1839; ORTEA & BACALLADO, 1981; PERRONE, 1989), while *Doris alboranica* Bouchet, 1977 also has hooked marginal teeth without denticles and a bursa copulatrix globose and much larger than the seminal receptacle which is pyriform (BOUCHET, 1977). In *Doris*

*pseudoargus* Rapp, 1827 the bursa copulatrix is irregular in shape, about 10 times larger than the seminal receptacle and shows a single large blood gland (VALDÉS, 2002). All of the above characters clearly differentiate these thirteen eastern North Atlantic species from *Doris adrianae* sp. nov., which corroborates the validity of this new species.

In general, the internal anatomy of *Doris adrianae* sp. nov. matches the diagnostic characters of the genus *Doris* established by VALDÉS (2002). However, in the description of the reproductive apparatus (*vide supra*) it is explained that in the final section of the distal oviduct, close to its opening in the genital atrium, there is a globular structure of glandular appearance that could be a vestibular gland, most likely as an adhesive region (Figs. 8G,H, 9H). This is the only character that does not coincide with the diagnosis of the genus *Doris*, in which VALDÉS (2002) states that vestibular or accessory glands absent. It is not a vestibular gland in its usual sense, which is very diverse, as it is usually described, sometimes as a clearly visible and prominent pouch, and sometimes as a small swelling, of diverse location in the genital opening, in the opening of the oviduct, on one side or at the end of the female glandular mass and with lobulated, branched, blind tubule-like forms, among others. This type of vestibular gland was widely mentioned in species of several families of the Infraorder Doridoidei, but it is considered that these glands are not homologous among these families (RUDMAN, 1985, 1987; GOSLINER, 1994; JOHNSON & GOSLINER, 1998; VALDÉS & CAMPILLO, 2000; VALDÉS, 2002).

WÄGELE (1985; 1989) described a specialised glandular tissue near the distal oviduct opening in *Phyllidia pulitzeri* Pruvot-Fol, 1962 and *Austrodoris kerguelenensis* (Bergh, 1884 in BERGH 1884b) which she refers to as the adhesive region or “Kleberregion”, a term already used by SCHMEKEL (1971). WÄGELE, *et al.* (1999) consider in *Dendrodoris nigra* that the function of the vestibular gland has more to do with egg laying than receipt of sperm and KLUSSMANN-KOLB & BRODIE (1999) who studied it histologically and ultrastructurally, observed that internally the gland itself presents

a characteristic convoluted appearance and that the position of the gland seems to reflect a specific function in the final stages of egg mass production.

KLUSSMANN-KOLB (1999; 2001a; 2001b) did histological and ultrastructural research on the nidamental glandular system in several heterobranchs sea slugs (Cephalaspidea, Aplysiida, Sacoglossa and Nudibranchia), describing in detail a specialised glandular epithelium lining the most distal part of the oviduct, consisting of columnar glandular cells with short to moderately long cilia, which he named adhesive region. The vestibular gland located in the distal oviduct of the genital tract of *Doris adrianae* sp. nov. with ciliated columnar cells (Fig. 9H) has remarkable similarities with the adhesive region described by KLUSSMANN-KOLB (2001b). Thus, considering that the adhesive region is frequent in several dorid species, because it is the part where the secretion of an adhesive substance that binds the egg masses to the substratum takes place, it must also be present in many other dorid species (WÄGELE, personal communication). However, a detailed histological and ultrastructural study of the nidamental gland of *Doris adrianae* sp. nov. will be necessary to determine with certainty that it is undoubtedly an adhesive region.

As explained, the egg mass of *Doris adrianae* sp. nov. is a festoon ribbon of 2 to 2.5 turns with one edge adhering to the substratum and with the upper edge brown due to the presence of fusiform spicules. In the scientific literature on nudibranchs (FERNANDEZ-OVIES & ORTEA, 1981; KLUSSMANN-KOLB & WÄGELE, 2001; WILSON, 2002; among others) no reference was found describing in any species the presence of spicules on the egg ribbon, which reveals another very specific character in *Doris adrianae* sp. nov.

As far as feeding is concerned, it is a known fact that poriferans are the basis of the diet of dorids. Apparently, the diet does not seem to be very selective, because in many occasions the sponges on which the animals are placed are considered as food. This fact should not be taken for granted, the most reliable way is

to analyse the spicules of the faecal pellets, which even allows us to obtain proportions in the case that the dorid feeds on more than one species of sponge. Thus, URGORRI & BESTEIRO (1984) by faecal pellet analysis obtained a proportion of spicules of 70% of *Hymeniacion perlevis* (Montagu, 1814) and 30% of *Halichondria panicea* (Pallas, 1766) in *Doris verrucosa*. In *Doris pseudoargus* 80% of the spicules of *Hymeniacion perlevis* (Montagu, 1814) and 20% of *Halichondria panicea* (Pallas, 1766) were obtained in one occasion and 100% of *Halichondria panicea* (Pallas, 1766) in another occasion.

Consequently, dorids do not feed on the species that are available in their environment, but rather they are located in those habitats where the species they prey on are present. To verify this fact, all the sponge species of large size present in the type locality were collected and identified (*vide supra* Habitat and associated fauna), in order to check the availability of potential food, including *Polymastia boletiformis* (Lamarck, 1815). Additionally, the faecal pellets of the holotype and 11 paratypes (4, 6, 8, 9, 13, 17, 18, 18, 19, 20, 21 and 22), the digestive contents of the dissected paratypes (1, 7, 10, 12 and 17) and the histological sections of paratype 15 were analysed. In all of them the result was the same, they contained only the tylostyles of *Polymastia boletiformis*.

In conclusion, it can be stated that *Doris adrianae* sp. nov. feeds exclusively on *Polymastia boletiformis* despite the presence of six other sponge species of good size in the environment in which it lives. It is also deduced that *Doris adrianae* sp. nov. is only found in habitats where *Polymastia boletiformis* is present, due to a monospecific predator-prey relationship, not appearing in other infralittoral bottoms of the Ría de Ferrol where several species of porifera are very abundant but where *Polymastia boletiformis* is not present, which determines the habitat where *Doris adrianae* sp. nov. is found.

Finally, as explained in the material and methods (*vide supra* DNA analysis), a total of 177 *cox1-5'* sequences within the genera *Doris*, *Archidoris* and *Austrodoris* were downloaded



from GenBank in December 2020, whose full list of downloaded sequences is provided in Supplementary material Appendix 1. *Doris adrianae* sp. nov. has a high genetic distance to other species of the genus *Doris* whose sequences are available in GenBank. However, this high genetic distance (approx. 20% of different nucleotides in the barcode fragment) is similar to that among other *Doris* species (except for the species pair *Doris nobilis* and *Doris pseudoargus*). The Bayesian tree constructed with Beast (Fig. 10), shows *Doris adrianae* sp. nov. as a sister species to the clade composed of *Doris wellingtonensis*, *Doris montereyensis*, *Doris pseudoargus* and *Doris nobilis*, although the support for this relationship is not high (Bayesian posterior probability=0.709). However, it should be noted that this phylogenetic analysis has been carried out on the basis of the sequences available in GenBank and, therefore, the diversity of the genus *Doris*, for which 51 species are recognised (WoRMS, 2021), is under-represented. Therefore, genetic distances could be smaller with other species that are not currently represented in GenBank.

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**Table 1.** Genetic distance matrix showing the uncorrected pairwise identity (i.e. proportion of identical sites in the barcode fragment) between sequences of *Doris adrianae* sp. nov. and other *Doris* species. Sequences have been downloaded from GenBank and one sequence has been selected per species (according to GenBank identity). GenBank accession numbers are also provided.

**Tabla 1.** Matriz de distancia genética que muestra la identidad por pares no corregida (es decir, la proporción de sitios idénticos en el fragmento del “barcode”) entre las secuencias de *Doris adrianae* sp. nov. y otras especies de *Doris*. Las secuencias se han descargado de GenBank y se ha seleccionado una secuencia por especie (según la identidad de GenBank). También se proporcionan los números de acceso de GenBank.

	<i>Doris adrianae</i> sp. nov.	<i>Doris</i> sp.	EU823145.1 <i>Austrodoris kerguelensis</i>	JX680585.1 <i>Doris kerguelensis</i>	AJ223256.1 <i>Archidoris pseudoargus</i>	KR084616.1 <i>Doris pseudoargus</i>	GQ292034.1 <i>Archidoris wellingtonensis</i>	GQ292041.1 <i>Archidoris montereyensis</i>	KF643613.1 <i>Doris montereyensis</i>	MG935354.1 <i>Doris nobilis</i>
<i>Doris adrianae</i> sp. nov.	100	78.3	78.9	80.2	82	82.6	80.3	81	80.9	82.8
<i>Doris</i> sp.	78.3	99.8	79.3	79.9	76.7	77	77.4	76.8	76.7	77.3
EU823145.1 <i>Austrodoris kerguelensis</i>	78.9	79.3	100	95.1	81.8	81.8	79.7	80.4	80.5	82.3
JX680585.1 <i>Doris kerguelensis</i>	80.2	79.9	95.1	100	82.3	82.1	80.2	81.3	81.3	82.6
AJ223256.1 <i>Archidoris pseudoargus</i>	82	76.7	81.8	82.3	99.2	98.5	84	86.6	86.2	99
KR084616.1 <i>Doris pseudoargus</i>	82.6	77	81.8	82.1	98.5	100	84.1	87.3	86.8	99.4
GQ292034.1 <i>Archidoris wellingtonensis</i>	80.3	77.4	79.7	80.2	84	84.1	100	84.6	83.3	84.6
GQ292041.1 <i>Archidoris montereyensis</i>	81	76.8	80.4	81.3	86.6	87.3	84.6	100	99.3	87.3
KF643613.1 <i>Doris montereyensis</i>	80.9	76.7	80.5	81.3	86.2	86.8	83.3	99.3	100	86.6
MG935354.1 <i>Doris nobilis</i>	82.8	77.3	82.3	82.6	99	99.4	84.6	87.3	86.6	100

**Appendix 1.** Accession number and identification of sequences downloaded from GenBank on December 2020.

**Apéndice 1.** Número de acceso e identificación de las secuencias descargadas de GenBank en diciembre de 2020.

GenBank Accession Number	Species	GenBank Accession Number	Species	GenBank Accession Number	Species
AJ223256.1	<i>Archidoris pseudoargus</i>	EU823185.1	<i>Austrodoris kerguelenensis</i>	JX680547.1	<i>Doris kerguelenensis</i>
EU823127.1	<i>Austrodoris kerguelenensis</i>	EU823186.1	<i>Austrodoris kerguelenensis</i>	JX680548.1	<i>Doris kerguelenensis</i>
EU823128.1	<i>Austrodoris kerguelenensis</i>	EU823187.1	<i>Austrodoris kerguelenensis</i>	JX680549.1	<i>Doris kerguelenensis</i>
EU823129.1	<i>Austrodoris kerguelenensis</i>	EU823188.1	<i>Austrodoris kerguelenensis</i>	JX680550.1	<i>Doris kerguelenensis</i>
EU823130.1	<i>Austrodoris kerguelenensis</i>	EU823189.1	<i>Austrodoris kerguelenensis</i>	JX680551.1	<i>Doris kerguelenensis</i>
EU823131.1	<i>Austrodoris kerguelenensis</i>	EU823190.1	<i>Austrodoris kerguelenensis</i>	JX680552.1	<i>Doris kerguelenensis</i>
EU823132.1	<i>Austrodoris kerguelenensis</i>	EU823191.1	<i>Austrodoris kerguelenensis</i>	JX680553.1	<i>Doris kerguelenensis</i>
EU823133.1	<i>Austrodoris kerguelenensis</i>	EU823192.1	<i>Austrodoris kerguelenensis</i>	JX680554.1	<i>Doris kerguelenensis</i>
EU823134.1	<i>Austrodoris kerguelenensis</i>	EU823193.1	<i>Austrodoris kerguelenensis</i>	JX680555.1	<i>Doris kerguelenensis</i>
EU823135.1	<i>Austrodoris kerguelenensis</i>	EU823194.1	<i>Austrodoris kerguelenensis</i>	JX680556.1	<i>Doris kerguelenensis</i>
EU823136.1	<i>Austrodoris kerguelenensis</i>	EU823195.1	<i>Austrodoris kerguelenensis</i>	JX680557.1	<i>Doris kerguelenensis</i>
EU823137.1	<i>Austrodoris kerguelenensis</i>	EU823196.1	<i>Austrodoris kerguelenensis</i>	JX680558.1	<i>Doris kerguelenensis</i>
EU823138.1	<i>Austrodoris kerguelenensis</i>	EU823197.1	<i>Austrodoris kerguelenensis</i>	JX680559.1	<i>Doris kerguelenensis</i>
EU823139.1	<i>Austrodoris kerguelenensis</i>	EU823198.1	<i>Austrodoris kerguelenensis</i>	JX680560.1	<i>Doris kerguelenensis</i>
EU823140.1	<i>Austrodoris kerguelenensis</i>	EU823199.1	<i>Austrodoris kerguelenensis</i>	JX680561.1	<i>Doris kerguelenensis</i>
EU823141.1	<i>Austrodoris kerguelenensis</i>	EU823200.1	<i>Austrodoris kerguelenensis</i>	JX680562.1	<i>Doris kerguelenensis</i>
EU823142.1	<i>Austrodoris kerguelenensis</i>	EU823201.1	<i>Austrodoris kerguelenensis</i>	JX680563.1	<i>Doris kerguelenensis</i>
EU823143.1	<i>Austrodoris kerguelenensis</i>	EU823202.1	<i>Austrodoris kerguelenensis</i>	JX680564.1	<i>Doris kerguelenensis</i>
EU823144.1	<i>Austrodoris kerguelenensis</i>	EU823203.1	<i>Austrodoris kerguelenensis</i>	JX680565.1	<i>Doris kerguelenensis</i>
EU823145.1	<i>Austrodoris kerguelenensis</i>	EU823204.1	<i>Austrodoris kerguelenensis</i>	JX680566.1	<i>Doris kerguelenensis</i>
EU823146.1	<i>Austrodoris kerguelenensis</i>	EU823205.1	<i>Austrodoris kerguelenensis</i>	JX680567.1	<i>Doris kerguelenensis</i>
EU823147.1	<i>Austrodoris kerguelenensis</i>	EU823206.1	<i>Austrodoris kerguelenensis</i>	JX680568.1	<i>Doris kerguelenensis</i>
EU823148.1	<i>Austrodoris kerguelenensis</i>	EU823207.1	<i>Austrodoris kerguelenensis</i>	JX680569.1	<i>Doris kerguelenensis</i>
EU823149.1	<i>Austrodoris kerguelenensis</i>	EU823208.1	<i>Austrodoris kerguelenensis</i>	JX680570.1	<i>Doris kerguelenensis</i>
EU823150.1	<i>Austrodoris kerguelenensis</i>	EU823209.1	<i>Austrodoris kerguelenensis</i>	JX680571.1	<i>Doris kerguelenensis</i>
EU823151.1	<i>Austrodoris kerguelenensis</i>	EU823210.1	<i>Austrodoris kerguelenensis</i>	JX680572.1	<i>Doris kerguelenensis</i>
EU823152.1	<i>Austrodoris kerguelenensis</i>	EU823211.1	<i>Austrodoris kerguelenensis</i>	JX680573.1	<i>Doris kerguelenensis</i>
EU823153.1	<i>Austrodoris kerguelenensis</i>	EU823212.1	<i>Austrodoris kerguelenensis</i>	JX680574.1	<i>Doris kerguelenensis</i>
EU823154.1	<i>Austrodoris kerguelenensis</i>	EU823213.1	<i>Austrodoris kerguelenensis</i>	JX680575.1	<i>Doris kerguelenensis</i>
EU823155.1	<i>Austrodoris kerguelenensis</i>	EU823214.1	<i>Austrodoris kerguelenensis</i>	JX680576.1	<i>Doris kerguelenensis</i>
EU823156.1	<i>Austrodoris kerguelenensis</i>	EU823215.1	<i>Austrodoris kerguelenensis</i>	JX680577.1	<i>Doris kerguelenensis</i>
EU823157.1	<i>Austrodoris kerguelenensis</i>	EU823216.1	<i>Austrodoris kerguelenensis</i>	JX680578.1	<i>Doris kerguelenensis</i>
EU823158.1	<i>Austrodoris kerguelenensis</i>	EU823217.1	<i>Austrodoris kerguelenensis</i>	JX680579.1	<i>Doris kerguelenensis</i>
EU823159.1	<i>Austrodoris kerguelenensis</i>	EU823218.1	<i>Austrodoris kerguelenensis</i>	JX680580.1	<i>Doris kerguelenensis</i>
EU823160.1	<i>Austrodoris kerguelenensis</i>	GQ292034.1	<i>Archidoris wellingtonensis</i>	JX680581.1	<i>Doris kerguelenensis</i>
EU823161.1	<i>Austrodoris kerguelenensis</i>	GQ292035.1	<i>Doris kerguelenensis</i>	JX680582.1	<i>Doris kerguelenensis</i>
EU823162.1	<i>Austrodoris kerguelenensis</i>	GQ292036.1	<i>Doris kerguelenensis</i>	JX680583.1	<i>Doris kerguelenensis</i>
EU823163.1	<i>Austrodoris kerguelenensis</i>	GQ292037.1	<i>Doris kerguelenensis</i>	JX680584.1	<i>Doris kerguelenensis</i>
EU823164.1	<i>Austrodoris kerguelenensis</i>	GQ292038.1	<i>Doris kerguelenensis</i>	JX680585.1	<i>Doris kerguelenensis</i>
EU823165.1	<i>Austrodoris kerguelenensis</i>	GQ292039.1	<i>Doris kerguelenensis</i>	JX680586.1	<i>Doris kerguelenensis</i>
EU823166.1	<i>Austrodoris kerguelenensis</i>	GQ292041.1	<i>Archidoris montereyensis</i>	JX680587.1	<i>Doris kerguelenensis</i>



## Apéndice 1. Continuación

EU823167.1	<i>Austrodoris kerguelenensis</i>	GQ292046.1	<i>Doris</i> sp.	JX680588.1	<i>Doris kerguelenensis</i>
EU823168.1	<i>Austrodoris kerguelenensis</i>	GU227115.1	<i>Austrodoris kerguelenensis</i>	JX680589.1	<i>Doris kerguelenensis</i>
EU823169.1	<i>Austrodoris kerguelenensis</i>	JX680531.1	<i>Doris kerguelenensis</i>	KC153022.1	<i>Doris montereyensis</i>
EU823170.1	<i>Austrodoris kerguelenensis</i>	JX680532.1	<i>Doris kerguelenensis</i>	KF643435.1	<i>Doris montereyensis</i>
EU823171.1	<i>Austrodoris kerguelenensis</i>	JX680533.1	<i>Doris kerguelenensis</i>	KF643446.1	<i>Doris montereyensis</i>
EU823172.1	<i>Austrodoris kerguelenensis</i>	JX680534.1	<i>Doris kerguelenensis</i>	KF643613.1	<i>Doris montereyensis</i>
EU823173.1	<i>Austrodoris kerguelenensis</i>	JX680535.1	<i>Doris kerguelenensis</i>	KF643914.1	<i>Doris montereyensis</i>
EU823174.1	<i>Austrodoris kerguelenensis</i>	JX680536.1	<i>Doris kerguelenensis</i>	KF644212.1	<i>Doris montereyensis</i>
EU823175.1	<i>Austrodoris kerguelenensis</i>	JX680537.1	<i>Doris kerguelenensis</i>	KR084378.1	<i>Doris pseudoargus</i>
EU823176.1	<i>Austrodoris kerguelenensis</i>	JX680538.1	<i>Doris kerguelenensis</i>	KR084586.1	<i>Doris pseudoargus</i>
EU823177.1	<i>Austrodoris kerguelenensis</i>	JX680539.1	<i>Doris kerguelenensis</i>	KR084616.1	<i>Doris pseudoargus</i>
EU823178.1	<i>Austrodoris kerguelenensis</i>	JX680540.1	<i>Doris kerguelenensis</i>	KR084907.1	<i>Doris pseudoargus</i>
EU823179.1	<i>Austrodoris kerguelenensis</i>	JX680541.1	<i>Doris kerguelenensis</i>	MF958425.1	<i>Doris montereyensis</i>
EU823180.1	<i>Austrodoris kerguelenensis</i>	JX680542.1	<i>Doris kerguelenensis</i>	MG422353.1	<i>Doris montereyensis</i>
EU823181.1	<i>Austrodoris kerguelenensis</i>	JX680543.1	<i>Doris kerguelenensis</i>	MG935320.1	<i>Doris pseudoargus</i>
EU823182.1	<i>Austrodoris kerguelenensis</i>	JX680544.1	<i>Doris kerguelenensis</i>	MG935354.1	<i>Doris nobilis</i>
EU823183.1	<i>Austrodoris kerguelenensis</i>	JX680545.1	<i>Doris kerguelenensis</i>	MG935407.1	<i>Doris pseudoargus</i>
EU823184.1	<i>Austrodoris kerguelenensis</i>	JX680546.1	<i>Doris kerguelenensis</i>	MH242734.1	<i>Doris montereyensis</i>