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Scientific articles

Redescription of *Algarvia alba* García-Gómez & Cervera, 1989 (Mollusca, Nudibranchia, Aeolidioidea) from the Ría de Ferrol (Galicia, NW Iberian Peninsula).

Redescrición de *Algarvia alba* García-Gómez & Cervera, 1989 (Mollusca, Nudibranchia, Aeolidioidea) da Ría de Ferrol (Galicia, NW Iberian Peninsula).

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Abstract

Algarvia alba García-Gómez & Cervera, 1989 is a little-known nudibranch mollusk (Aeolidioidea: Facelinidae) that inhabits sandy-rocky substrates along the Atlantic coast of the Iberian Peninsula. This species is only known from 3 specimens, two corresponding to the type series and collected in Algarve (Portugal), and one specimen photographed in Getaria (Basque Country). The anatomy of a new specimen collected from the Ría de Ferrol is described in detail for the first time by means of X-ray microcomputed tomography (Micro-CT); this non-destructive technique allows obtaining sequential images of the sagittal, frontal and transverse planes. The systematics of Facelinidae is not properly resolved yet and therefore we propose to keep *Algarvia* in this family until more information becomes available.

Keywords: Gastropoda; Nudibranchia; Ría de Ferrol; anatomy; habitat; X-ray computing microtomography.

Resumo

Algarvia alba García-Gómez & Cervera, 1989 é un molusco nudibranquio pouco coñecido (Aeolidioidea: Facelinidae) que habita en substratos areoso-rochosos ao longo da costa atlántica da Península Ibérica. Esta especie só se coñece a partir de 3 exemplares, dous correspondentes á serie tipo e recollidos no Algarve (Portugal), e un exemplar fotografado en Getaria (País Vasco). Descríbese por primeira vez en detalle a anatomía dun novo exemplar recollido na Ría de Ferrol mediante microtomografía computarizada de raios X (Micro-TC); esta técnica non destrutiva permite obter imaxes secuenciais dos planos saxital, frontal e transversal. A sistemática de Facelinidae aínda non está debidamente resolta e, polo tanto, propoñemos manter o Algarvia nesta familia ata que se dispoña de máis información.

Palavras chave: Gastropoda; Nudibranchia; Ría de Ferrol; anatomía; hábitat; microtomografía computarizada.

Abbreviations

a:	anus	**ga:**	genital atrium	**ov:**	oviduct
am:	ampulla	**gb:**	buccal ganglion	**pe:**	penis
au:	auricle	**gc:**	capsule gland	**pg:**	penial gland
bb:	bucal bulb	**gcp:**	cerebropleural ganglion	**pr:**	pericardium
bc:	bursa copulatrix	**gm:**	membrane gland	**pr1:**	prostate1
cb:	cerebropleural-buccal connective	**gmu:**	mucous gland	**pr2:**	prostate 2
cf:	fertilization chamber	**gp:**	pedal ganglion	**pt:**	propodeal tentacles
cn:	cnidosac	**gpo:**	postampullar gonoduct	**ra:**	radula
cp:	pedal commissure	**gpr:**	preampullar gonoduct	**rh:**	rhinophores
cr:	renal chamber	**gr:**	rhinophore ganglion	**sg:**	salivary gland
cy:	statocyst	**h:**	heart	**st:**	stomach
d:	deferent duct	**hg:**	hermaphrodite gland	**sy:**	syrinx
dg:	digestive gland	**in:**	intestine	**to:**	oral tentacles
dgl:	digestive gland left branch	**ja:**	jaw	**va:**	afferent vessel
dgr:	digestive gland right branch	**m:**	mouth	**vav:**	auricle-ventricular valve
es:	penial stylet	**ne:**	nephrostoma	**ve:**	efferent vessel
ey:	eye	**nep:**	nephroproct	**ven:**	ventricle
fg:	female glands	**oe:**	esophagus	**vg:**	vestibular gland
fo:	gonadal follicle	**og:**	oral glands	**vp:**	penial sheath
ft:	foot	**ot:**	oral tube		

INTRODUCTION

The suborder Cladobranchia (Mollusca: Gastropoda: Nudibranchia) includes several clades that display remarkable morphological and ethological diversity (Putz *et al.*, 2010; Goodheart, 2017). Among them, the family Facelinidae *sensu lato* stands out as a diverse group of predatory organisms that are quite mobile and generally display aggressive behavior (Thompson, 1988). Recently the monophyly of this family has been questioned (e.g., Carmona *et al.*, 2015; Goodheart *et al.*, 2017; Martynov *et al.*, 2019), and at the moment there is no general consensus regarding the classification of the different groups.

The genus *Algarvia* García-Gómez & Cervera, 1989 and its only known species, *Algarvia alba* García-Gómez & Cervera, 1989, was originally described from Sagres, on the southern coast of Portugal and classified in the family Facelinidae by García-Gómez & Cervera (1989) and Cervera *et al.* (2004). The description by García-Gómez & Cervera (1989) was based on two specimens (5 mm and 2.5 mm long respectively) but partly imprecise and limited with regards to the internal anatomy, as it only provided data on the radula, jaws and a partial description of the reproductive system. After the original description, only one additional specimen of *Algarvia alba* has been reported based on photographs taken on the coast of the Euskadi, in

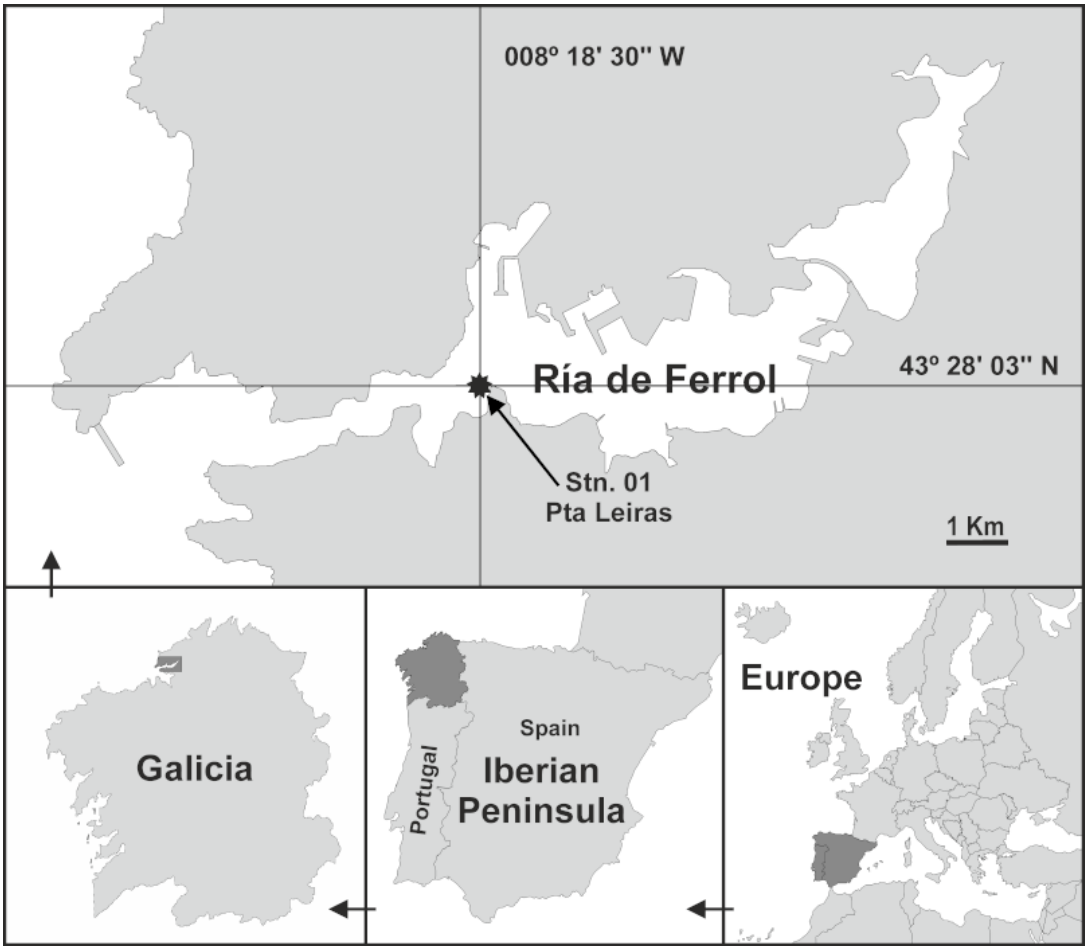
Getaria, Gipuzkoa, Spain (Silva *et al.*, 2016); this specimen was not collected neither examined. In 2009, staff of the Estación de Bioloxía Mariña da Graña collected a specimen of *Algarvia alba* on a subtidal sandy bottom 20 m deep at Punta Leiras in the Ría de Ferrol (Galicia). The anatomy of this specimen was described in detail using a non-destructive methodology, i.e. X-ray computed tomography (=Micro-CT), in order to avoid permanent damage, and presented herein.

MATERIAL AND METHODS

Collection: The studied specimen of *Algarvia alba* García-Gómez & Cervera, 1989 (identification code: 01110109), was collected on January 11, 2009, from Punta Leiras (Ría de Ferrol), at 20 m depth, on a bottom of medium-sand with some flat rocky outcrops, at the inner end of the ría entrance channel (43°28'03"N; 008°15'30"W) (Fig. 1). This area is subject to strong tidal currents. The collection of the sand sample was carried out by indirect sampling using a rectangular naturalist dredge, operated from a boat equipped with GPS positioning. The medium sand sample was transported to the laboratory for sorting of the infauna. Subsequently, several additional samples of medium sand were taken in the same locality but these did not yield further specimens of *Algarvia alba*.

Figure 1. Map showing the locality where the studied specimen of *Algarvia alba* was collected, Punta Leiras in the Ría de Ferrol (NW Iberian Peninsula).

Figura 1. Mapa da localidade onde recolleuse o exemplar estudado de *Algarvia alba* en Punta Leiras na Ría de Ferrol (NW Península Ibérica).



The specimen is deposited at the collection of the Museo de Historia Natural of the Universidade de Santiago de Compostela with the collection number: MHN USC-25102. The collected specimen (identification code: 01110109), measured *in vivo* 5 mm in length and 2 mm in width in the widest part, right behind the rhinophores; the width of the head was 1.3 mm and the oral tentacles 1 mm in length.

Separation and description *in vivo*: The specimen of *Algarvia alba* was separated from the sediment by allowing it to crawl to the surface. After collection, the animal was observed *in vivo* with an Olympus SZX12 stereoscopic microscope, describing all morphological characters, behaviors, and color patterns that are perishable after fixation. The animal *habitus* and details of body parts were photographed *in vivo* with an Olympus Camedia C-5050 Zoom digital camera, associated with an Olympus SZX12 stereoscopic microscope.

Anesthesia, fixation, and preservation: After the description, the animal was anesthetized with 7% $MgCl_2$, fixed in Bouin's fluid, and preserved in 70º ethanol buffered with borax for subsequent study by X-ray microcomputed tomography (Micro-CT).

DNA analysis: To perform barcode identification and confirm the correct taxonomic position of this species and to conduct molecular phylogenetic analyses to determine its evolutionary relationships, molecular studies were conducted. Although DNA extraction was performed, but it was not possible to amplify any DNA fragments, most likely due to the fixation of the specimen in Bouin's fluid which is the most suitable for X-ray computed microtomography (Micro-CT).

Micro-CT & AVIZO 6.4: X-ray computed microtomography (Micro-CT) is a non-invasive technique that allows to examine the anatomy of the specimen without altering or destroying the animal. The specimen of *Algarvia alba* (01110109) preserved in 70% ethanol was dehydrated in successive baths of 80%, 90% and 96% ethanol; then it was stained for three days in a 1% iodine solution in 96º ethanol, immersed in hexamethyldisilazane (HMDS) and air-dried overnight (Alba-Tercedor & Sánchez-Tocino, 2011; Faulwetter *et al.*, 2013; Candás *et al.*, 2016). The scans were performed with a Skyscan 1172 microtomograph (Bruker, Belgium) that allows obtaining 2D and 3D images of the entire anatomy of the animal, both external and internal. The obtained X-ray projection images were reconstructed with the NRecon software (Bruker, Belgium) and the sections were processed with the CTAn software (Bruker, Belgium), the Data Viewer software (Bruker, Belgium) and the CTVox software (Bruker, Belgium) that was used to obtain the 3D representations (Figs. 3A-C,E,F; 4C-E,G). The study of the images of the sections in the three planes (transverse, frontal, and sagittal) (Figs. 5C-J; 6D-I; 7C-E; 8A-H; 9B-D,F-K), facilitates the recognition of the different structures that make up the different systems of the specimen.

Once the sections were obtained, the reconstruction was carried out using the AVIZO 6.4 program, which produces three-dimensional anatomical models from two-dimensional images. After choosing the pixel size (1.28 µm) and loading the cross-sectional images in AVIZO, the images were aligned and the 3D reconstruction was conducted manually, drawing the different structures with the tools provided by the program; sometimes it was necessary to smooth them to eliminate imperfections and result in a sharper three-dimensional image (Figs. 5B; 7A; 9E,I). Through this process, a three-dimensional model that accurately reproduces the anatomy and connections between the different organs and apparatuses was obtained. Once the 3D model was obtained, it could be rotated and add or separate different structures to facilitate observation from any perspective.

RESULTS

Phylum **Mollusca** Linnaeus, 1758
Class **Gastropoda** Cuvier, 1795
Subclass **Heterobranchia** Burmeister, 1837
Infraclass **Euthyneura** Spengel, 1881
Superorder **Nudipleura** Wägele & Willan, 2000
Order **Nudibranchia** Cuvier, 1817
Suborder **Dexiarchia** Schrödl, Wägele & Willan, 2001
Superfamily **Aeolidioidea** Gray, 1827
Family **Facelinidae** Bergh, 1889
Genus ***Algarvia*** García-Gómez & Cervera, 1989
***Algarvia alba* García-Gómez & Cervera, 1989**

Material examined: 1 specimen (MHN USC-25102) 5 mm in length and 2 mm in width; head width: 1.3 mm, oral tentacles length: 1 mm. Punta Leiras, channel of the Ría de Ferrol (NW Iberian Peninsula), 20 m depth, medium sand bottom with some flat rocky outcrops (Fig. 1).

DESCRIPTION

Habitus: The body is small, elongated and limaciform in shape, milky white in color (Figs. 2A,B,G), except for the upper half of the rhinophores, veil, and oral tentacles, which are orange in color: RGB 225 -82-12 (Figs. 2A-C,E,F). Body covered with cerata arranged in dorsolateral bundles (Figs 2A; 3A; 4F). Head with a trapezoidal anterior cephalic veil, with the elongated, conical, and pointed oral tentacles emerging from the anterolateral ends (Figs. 2A,B,E,F). The lateral edges of the velum, behind the oral tentacles, possess a knotted edge formed by 15 tubercles (Fig. 2E) that are also present on the front edge, between the oral tentacles, but are less prominent. The veil covers the buccal area, which is prominent and rounded, and has the mouth located at the center (Figs. 2A,D,F; 3A,E). The cephalic veil is hyaline white, presenting an orange band on the anterior and lateral edges. This color band continues to the middle of the oral tentacles, whereas the rest of the tentacles are translucent white; the edging continues on their rear downwards surrounding the buccal area posteriorly, but not joining ventrally (Figs. 2A,B,E). The buccal area is translucent white and when the animal moves, it clearly protrudes, raising the cephalic veil and positioning the oral tentacles as if they were horns, in an apparently aggressive attitude (Figs. 2A,F; 3A).

The head narrows behind the oral veil, after which a pair of rhinophores emerge at the level of the first pair of the ceratal bundles (Figs. 2A-C,F; 3A-D). The rhinophores are slightly longer than the oral tentacles and are basally milky white, subapically orange, and apically semitransparent with white dots, thus displaying a gradient from basal milky white to subapical orange (Figs 2B,C,F).

Figure 2. *Habitus* and external appearance *in vivo of Algarvia alba* (Ría de Ferrol - 01110109). A: General view of the body in dorsal view. B: Lateral view of the head showing the eyes. C: Rhinophores. D: Buccal area, mouth, propodium and propodial tentacles in ventral view. E: Cephalic veil and oral tentacles. F: Semilateral cephalic view with protruding buccal area. G: General appearance in ventral view. (A-G: *in vivo*)

Figura 2. *Habitus* e aparencia externa *in vivo* de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Aspecto xeral en visión dorsal. **B**: Aspecto lateral cefálico no que aprécianse os ollos. **C**: Rinóforos. **D**: Morro, boca, propodio e tentáculos propodiais en visión ventral. **E**: Veo cefálico e tentáculos orais. **F**: Aspecto semilateral cefálico co morro protraído. **G**: Aspecto xeral en visión ventral. (A-G: *in vivo*).

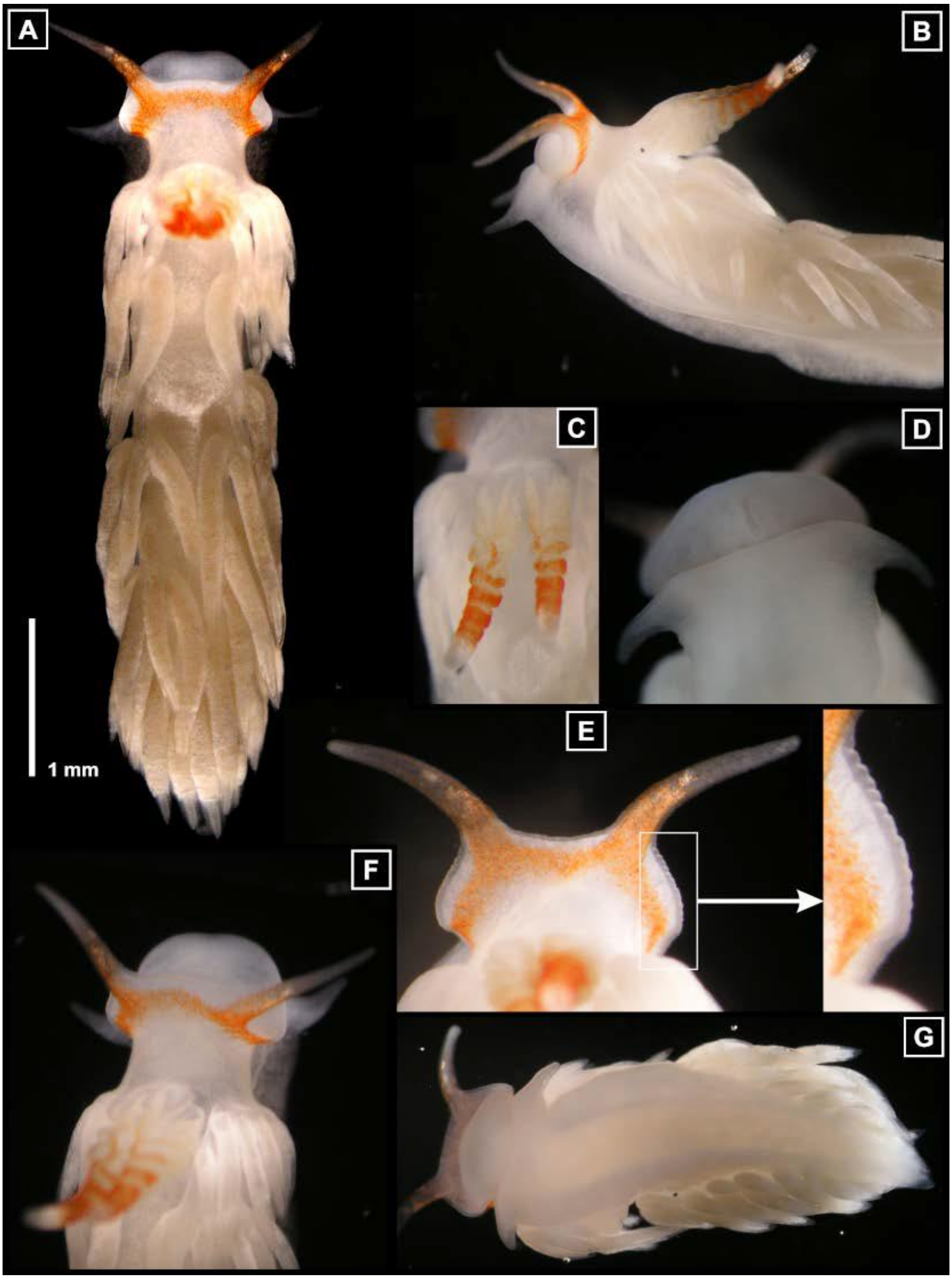
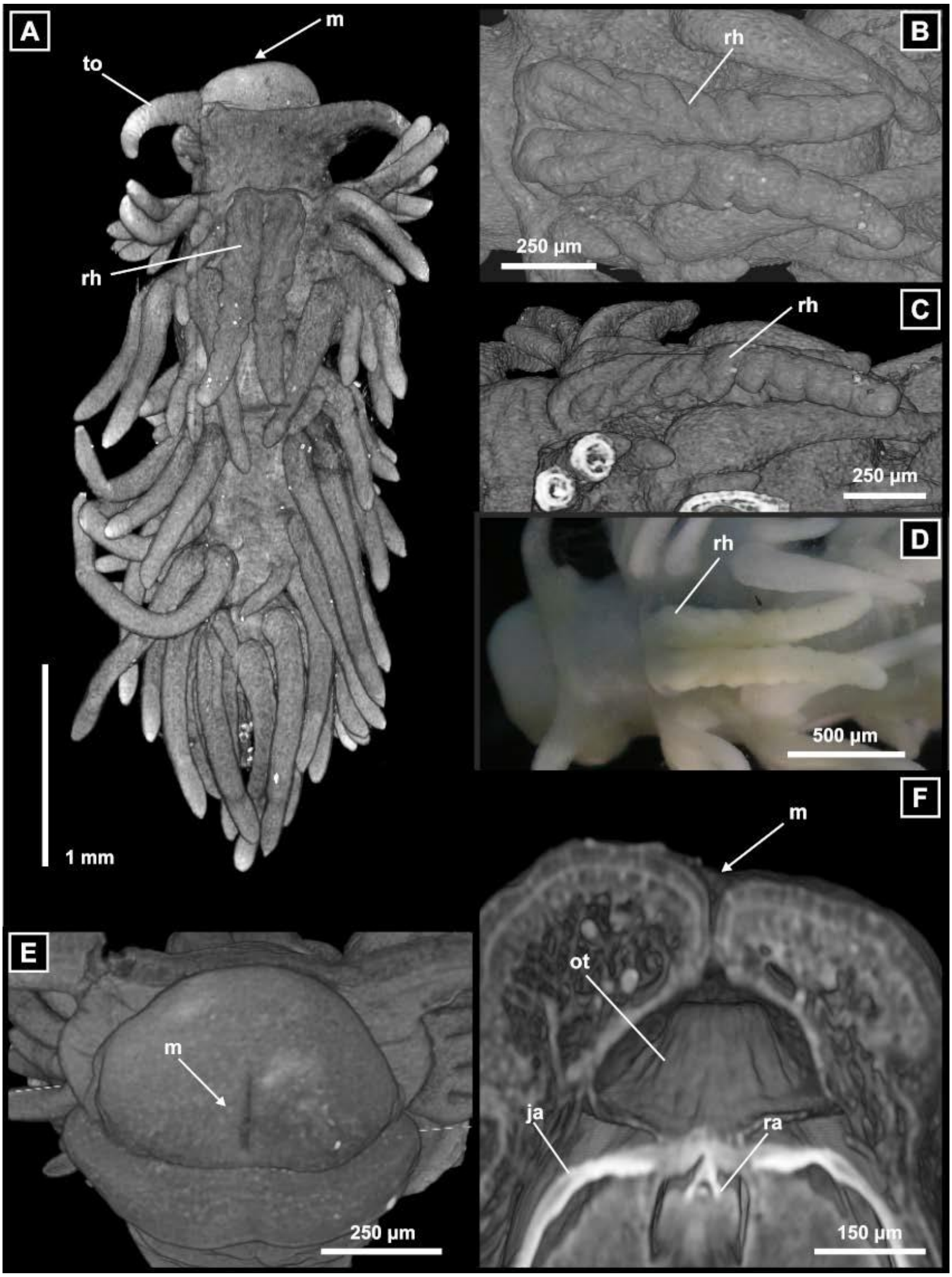


Figure 3. Anatomy of the preserved specimen of *Algarvia alba* (Ría de Ferrol - 01110109). A: 3D dorsal reconstruction of the animal. B: 3D dorsal reconstruction of the rhinophores. C: 3D lateral reconstruction of the rhinophores. D: Rhinophores in dorsal view. E: Frontal view of the buccal areas. F: Oral tube inside the oral cavity. (A,B,C,E,F: Micro-CT. D: OM).

Figura 3. Anatomía do exemplar conservado de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Reconstrución dorsal en 3D do animal. **B**: Reconstrución dorsal en 3D dos rinóforos. **C**: Reconstrución lateral en 3D dos rinóforos. **D**: Rinóforos en visión dorsal. **E**: Visión frontal do morro do animal. **F**: Tubo oral no interior da cavidade bucal. (A,B,C,E,F: Micro-CT. D: OM).



The rhinophores are 1.5 mm in length, emerging very close to each other and oriented obliquely backwards with the distal ends slightly curved upwards; when the animal is at rest the rhinophores are usually very close together. The rhinophores are club-shaped, with a white club and an orange, narrow stem (Figs. 2B,C); they are not smooth, since they possess thickenings with a semicircular section similar to knots, nine in number that are distributed obliquely from the external to the internal edge. The strong obliquity of the basal chord, almost vertical, decreases in the successive chords until the two ends are arranged like a ring (Figs. 2B,C; 3A-D). When the animal contracts the rhinophores, the obliquity becomes less apparent and the cords take on a more hoop-like appearance, especially the five upper ones, while when they are highly stretched, it is when the greatest obliquity is manifested, being appreciated in the furrows that separate the cords. The white pigment is not observed when the rhinophores are contracted, as the orange rings come together obscuring the white areas (Fig. 2C). At the base of each rhinophore, at the level of the outer edge, are the small eyes that can only be seen externally in lateral vision, never dorsal (Fig. 2B).

Most of the body is covered by two dorsolateral series of cerata bundles (Fig. 2A; 3A; 4C-E), nine bundles on each body side, with a different number of cerata on each one. The ceratal formula is:

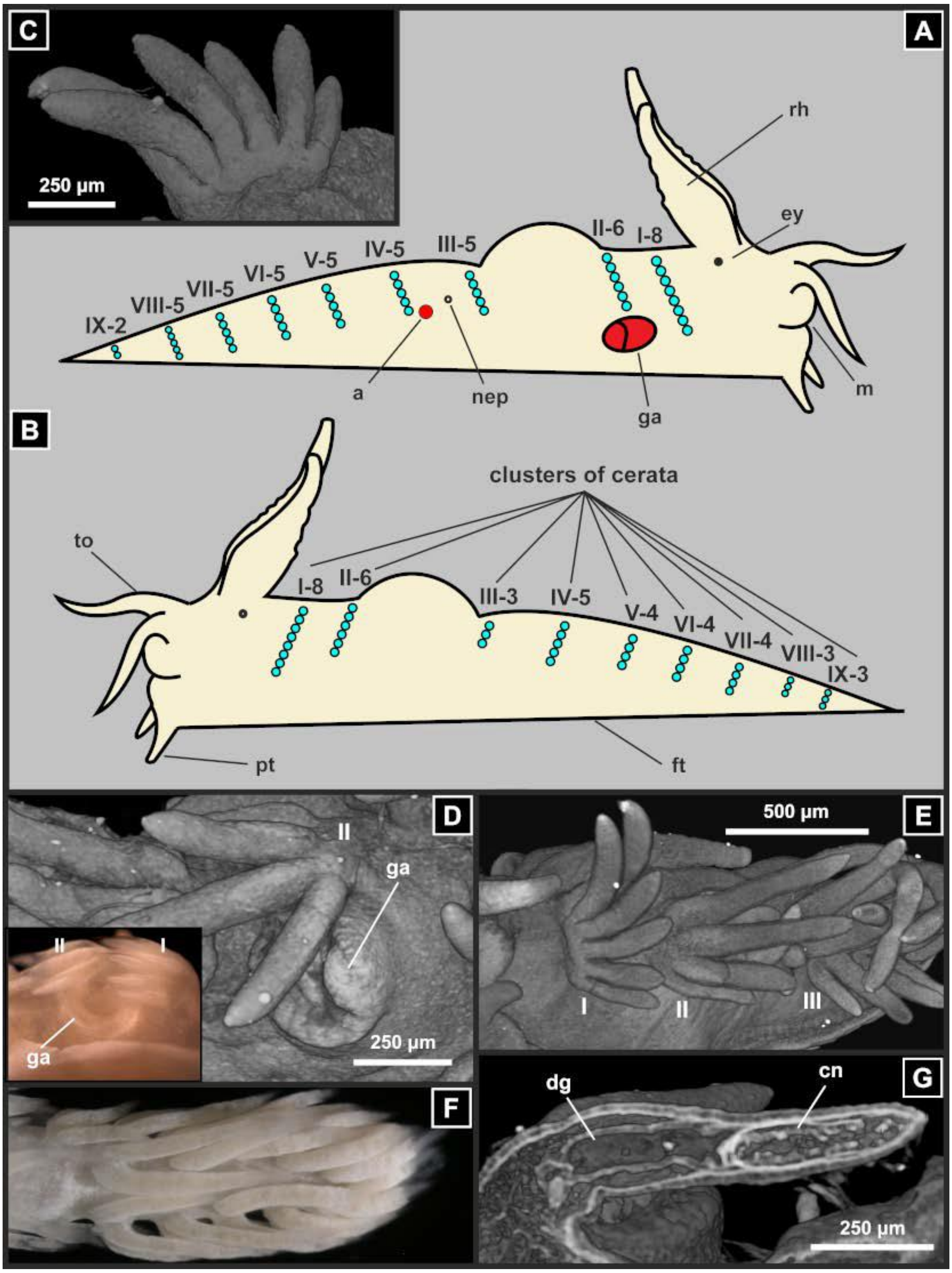
Ceratal bundles	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**Total I-IX**	
Nº cerata right lateral	**8**	**6**	**5**	**5**	**5**	**5**	**5**	**5**	**2**	**46**	**86**
Nº cerata left lateral	**8**	**6**	**3**	**5**	**4**	**4**	**4**	**3**	**3**	**40**	

In each bundle, the cerata are arranged in slightly oblique lines, the largest being the superior, medial ones, and the smallest the inferior, lateral ones (Fig. 4E). The location of the first bundles begins at the level of the anterior part of the rhinophores (Figs. 2A, 3A). The ceratal bundles of I and II are prepericardial and in equal numbers on both sides, while the rest (bundles III to IX) are postpericardial (Figs. 4A,B), the number on the right side being different from those on the left side. There are 46 cerata on the right side and 40 on the left. The cerata cover the entire dorsum and sides of the animal, with the exception of the pericardial area, posterior to the rhinophores (Figs. 2A; 4A-B-F). The cerata are spindle-shaped, but when the living animal moves, they are cylindrical for most of their length (Fig. 2A). The cnidosacs are large (Fig. 4G), clearly visible in the live animal (Fig. 2A). When the animal is disturbed by touching its head, it bristles the cerata, directing the first beams mainly forwards.

The genital atrium opening in the living animal was highly dilated, located just below the second ceratal bundle on the right side (Figs. 4A,D). The anus is cleioproctic, opening in the interceratal space between the III and IV right ceratal bundles, but very close to the base of the IV bundle (Fig. 4A). The nephroproct is also postpericardial, opening into the same interceratal space as the anus, but medially (Fig. 4A).

Figure 4. *Algarvia alba* (Ría de Ferrol - 01110109). A: Distribution of the ceratal bundles, tentacles and natural holes on the right side of the specimen. B: Distribution of the ceratal bundles and tentacles on the left side of the specimen. C: First left ceratal bundle. D: Genital atrium in preserved and *in vivo* (insert, bottom left) animal. E: First bundles of cerata on the left side. F: Postpericardial dorsal arrangement of the cerata *in vivo*. G: Cnidosac in longitudinal section of a ceras (C,D,E,G: Micro-CT. D,F: *in vivo*).

Figura 4. *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Distribución dos feixes de ceratas, tentáculos e furados naturais no lado dereito do exemplar. **B**: Distribución dos feixes de ceratas e tentáculos do lado esquerdo do exemplar. **C**: Primeiro feixe de ceratas esquerdo. **D**: Atrio xenital no animal conservado e *in vivo* (recadro, esquerda abaixo). **E**: Primeiros feixes de ceratas do lado esquerdo. **F**: Disposición dorsal postpericárdica dos ceratas *in vivo*. **G**: Cnidosaco en sección lonxitudinal dun cerata (C,D,E,G: Micro-CT. D,F: *in vivo*).



A broad foot occupies the entire ventral side of the animal (Fig. 2G), with the short, conical, sharp propodial tentacles located at anterolateral ends. The propodial tentacles are directed backwards when the animal is at rest, and recurved forward when the animal is moving (Figs. 2D,F,G). The anterior edge of the foot presents a very slight notch that appears to be more functional than morphological, since it can only be seen when the animal is at rest. The metapodium is slightly pointed, not exceeding the end of the last cerata. Neither the metapodium nor the lateral edges of the foot can be seen dorsally, only the propodial tentacles (Fig. 2A). The surface of the foot is white, with slightly yellowish hue observed in some central areas, probably due to the transparency of the viscera (Fig. 2G).

Internal Anatomy

The general description of the anatomy of the digestive and reproductive systems and of the nervous and renal pericardial systems is based on and extracted from 3D reconstructions.

Digestive system: The digestive tube opens to the outside anteriorly through a small mouth, located antero-ventrally at the end of the buccal area. The oral tube is located inside the oral cavity (Figs. 5G,H). A pair of elongated oral glands with a clustered appearance open at the base and extend from the base of the oral tube to the level of the beginning of the esophagus (Figs. 5A,B,E).

The buccal bulb is oval in shape, containing a pair of prominent jaws, with the masticatory edge apparently not denticulated and provided with powerful muscles (Figs. 6B-I). The radular sac is located medially in the buccal bulb (Figs. 6B-I), containing a uniseriate radula (29 x 0-1-0), formed by 29 denticulate teeth, 19 located in the upper part of the radular tape and 10 at the bottom (Figs. 6D,H). The denticulation of each tooth consists of a prominent central cusp and four small denticles on each side (Fig. 6A). A short esophagus opens on the posterior part of the buccal bulb (Figs. 5A,B) and is connected junction with a pair of sac-shaped salivary glands, that are shorter than the glands, (Figs. 5A,B,F).

Figure 5. Digestive system of *Algarvia alba* (Ría de Ferrol - 01110109). A: Schematic reconstruction of the regionalization of the digestive system. B: 3D schematic reconstruction of the digestive system. C: Postpericardial longitudinal section of the digestive gland. D: Transverse section of the digestive gland in lateral branches. E: Oral glands and their openings in the oral tube (arrows). F: Salivary gland and its opening into the esophagus (arrow). G-H: Oral tube inside the oral cavity and cross section (arrow). I-J: Cross section of the stomach, intestine and anus. (B: AVIZO 6.4. C-J: Micro-CT).

Figura 5. Aparato dixestivo de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Reconstrución esquemática da rexionalización do aparato dixestivo. **B**: Reconstrución esquemática en 3D do aparato dixestivo. **C**: Tramo lonxitudinal postpericárdico da glándula dixestiva. **D**: Sección transversal da glándula dixestiva en ramas laterais. **E**: Glándulas orais e a súa abertura no tubo oral (frechas). **F**: Glándula salivar e a súa abertura no esófago (frecha). **G-H**: Tubo oral no interior da cavidade bucal e sección transversal (frecha). **I-J**: Sección transversal do estómago, intestino e ano. (B: AVIZO 6.4. C-J: Micro-CT).

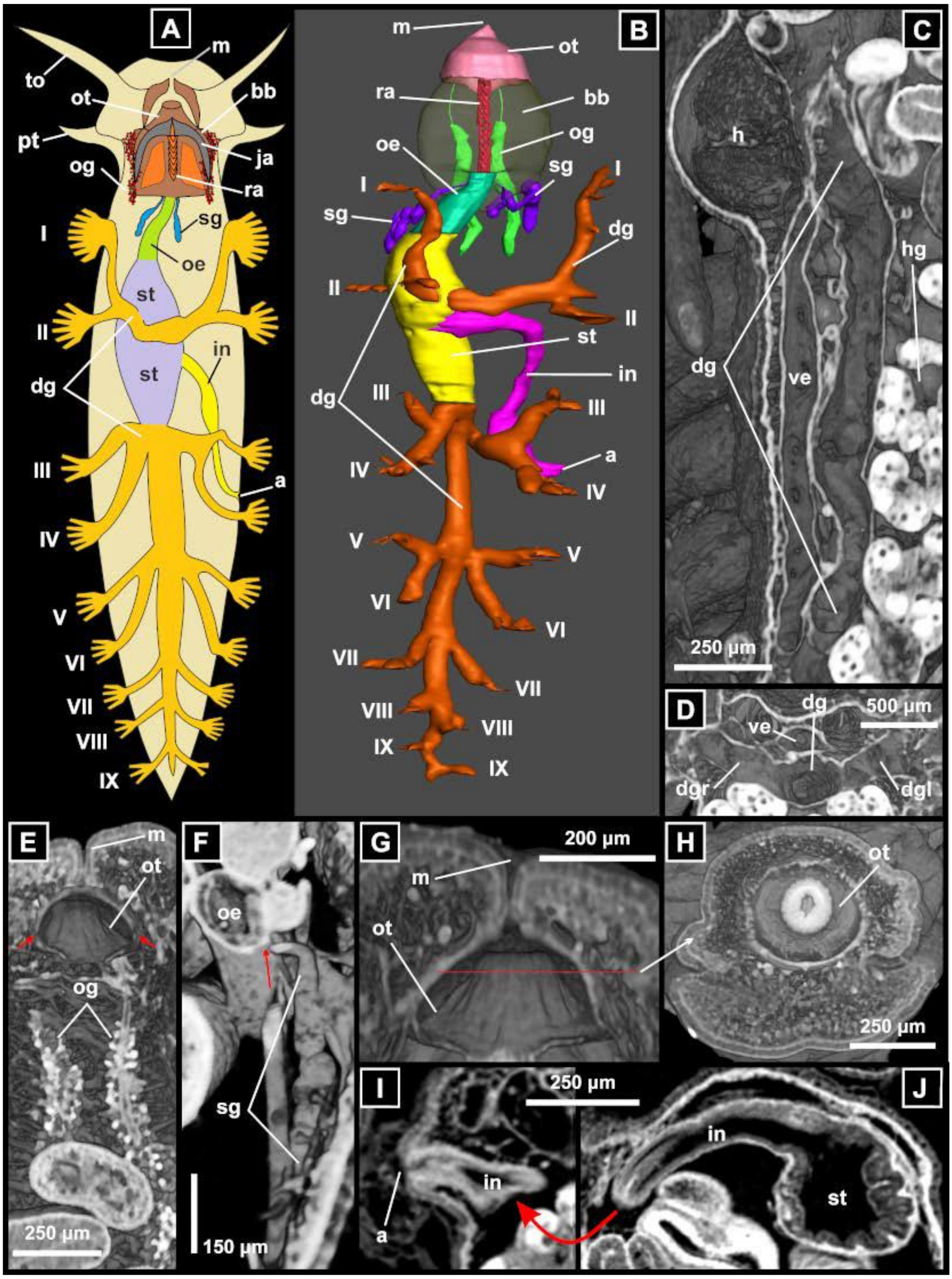
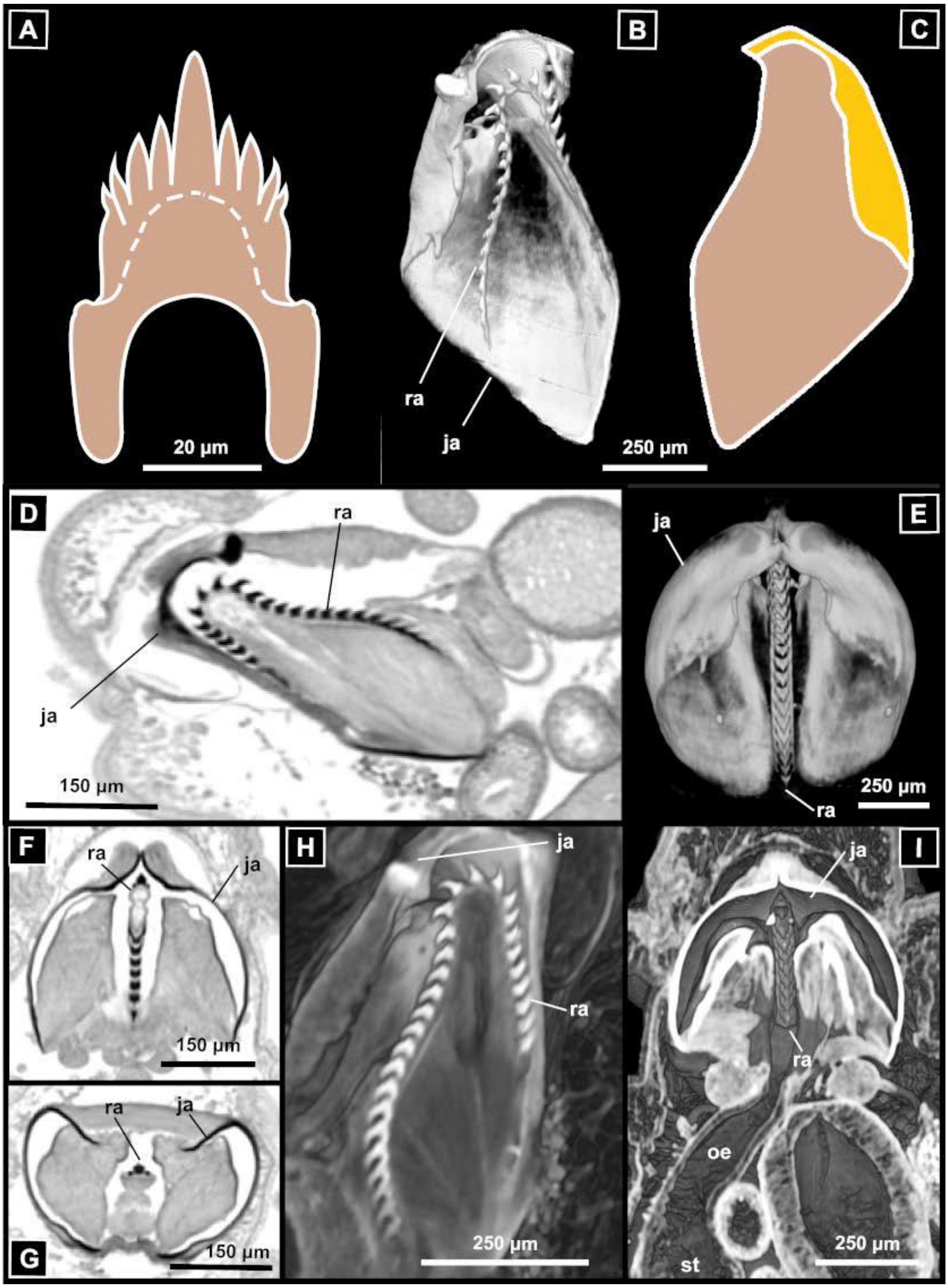


Figure 6. Digestive system: radula and jaws from *Algarvia alba* (Ría de Ferrol - 01110109). A: Schematic reconstruction of the radular tooth. B: Lateral view of the radula and jaw in 3D. C: Schematic reconstruction of the jaw. D-H: Radular band in median longitudinal section of the buccal bulb. E: Radula and jaws in 3D. F-G-I: Cross sections of the buccal bulb. (B,D-I: Micro-CT).

Figura 6. Aparato dixestivo: rádula e mandíbulas de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Reconstrución esquemática do dente radular. **B**: Visión lateral da rádula e mandíbula en 3D. **C**: Reconstrución esquemática da mandíbula. **D-H**: Fita radular en sección lonxitudinal medial do bulbo bucal. **E**: Rádula e mandíbulas en 3D. **F-G-I**: Secciόns transversais do bulbo bucal. (B,D-I: Micro-CT).



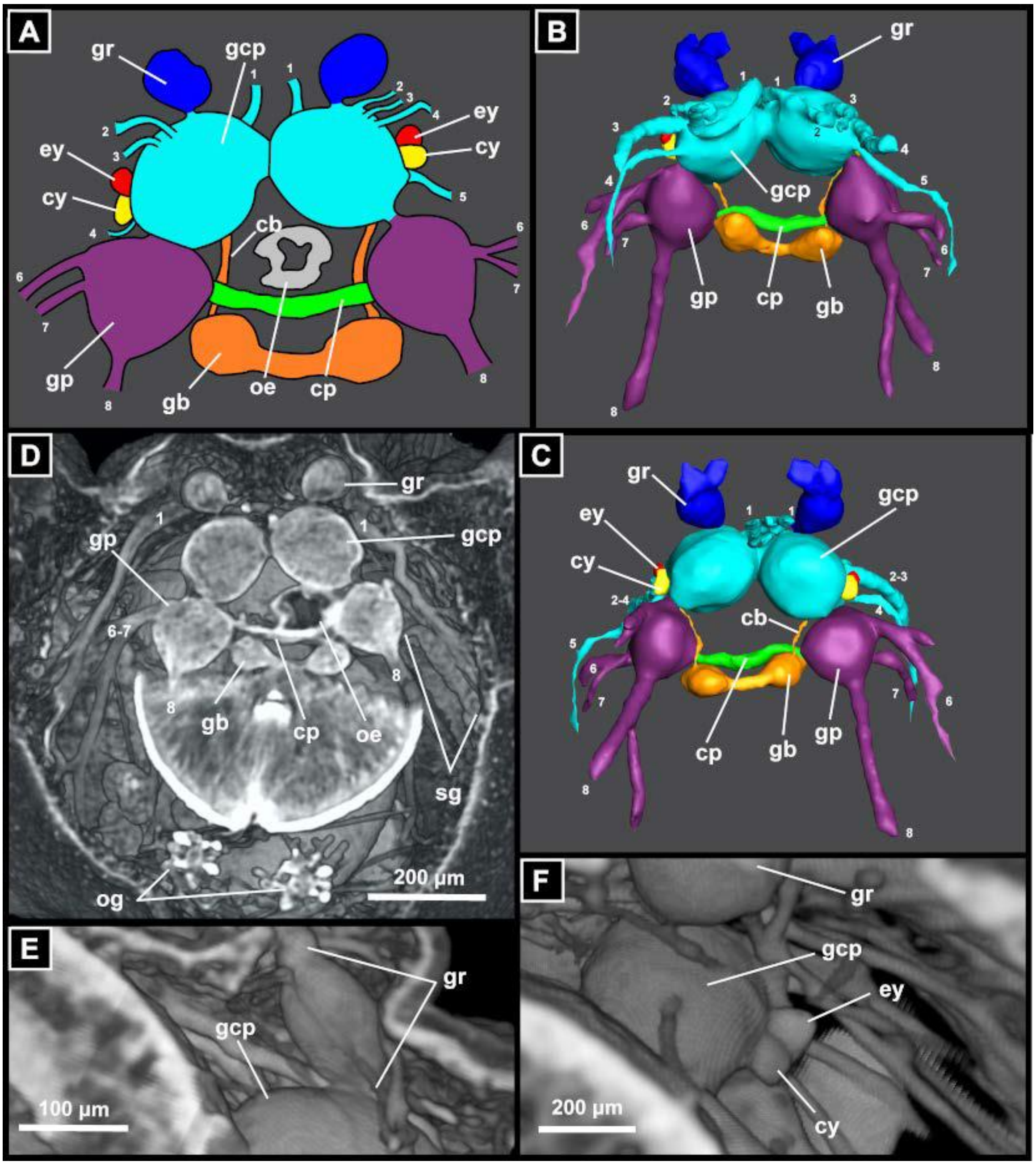
The esophagus opens into the stomach, which is well differentiated by the presence of gastric epithelial folds (Figs. 5A,B,J) and is displaced to the left side of the body, as a consequence of the presence of the reproductive system on the right side of the body. In The opening of the digestive gland originates in the anterior half of the stomach and then bifurcates on each side and leading into the precardiac ceratal bundles I and II (Figs. 5A,B). A third longitudinal section of the digestive gland originates on the posterior region of the stomach, (Figs. 5A,B); this section is, much longer and larger than the previous ones, running posteriorly along the mid-line of the body cavity, below the stomach afferent vessel and above the hermaphrodite gland (Figs. 5C,D) and emits lateral branches to the postpericardial cerata bundles (III-IX). The anterior branches (III to VI) originate from the longitudinal section in a bifurcated manner, while each of the three posterior branches (VII to IX) arises directly from the longitudinal section of the digestive gland (Figs. 5A,B). All branches of the digestive gland run inside the cerata to the base of the apical cnidosac. The cnidosacs in the distal part of the cerata are clearly visible in the live and preserved animal, although the nematocysts were not noticeable (Fig. 3G). The intestine arises transversely from the medial lateral part of the stomach, and opens to the exterior through the anus, which is located in the first postpericardiac interceratal space, just anterior to the base of the right IV ceratal bundle (Figs. 4A; 5A,B,I,J; 8H).

Nervous system: The nervous system is made up of a circumenteric ring composed by four pairs of ganglia: cerebropleural, pedal, rhinophoric, and buccal that are arranged in an annular fashion surrounding the esophagus (Figs. 7A-D).

The cerebropleural ganglia are slightly larger than the pedal ganglia and are located on the upper half of the circumenteric ring, joined by a short cerebropleural connective (Figs. 7A,B,C,D). The cerebropleural ganglia have five pairs of nerves, four of them exit laterally towards the cephalic part (Figs. 7A,B,C: 2,3,4,5) connecting with the jaw and foot muscles, while the fifth pair (Fig. 7A,B,C: 1) originates from the posteriodorsal part of the ganglion, running posteriorly to connect with the visceral mass. The small, well-developed eyes are located laterally on the cerebropleural ganglia, close to the pedal ganglia (Fig. 7A,B,C,F). The statocyst is located immediately behind the eyes in the cerebropleural ganglia (Fig. 7A, B,C,F). The pedal ganglia are located ventrally to the cerebropleural ganglia and lateral to the anterior esophagus and are attached ventrally by an apparent pedal commissure (Figs. 7A,B,C,D). Three pairs of nerves arise from the pedal ganglia, most of which connect to the pedal region (Fig. 7A,B,C,D: 6,7,8).

Figure 7. Nervous system of *Algarvia alba* (Ría de Ferrol - 01110109). A: Schematic reconstruction of the nervous system. B: 3D reconstruction of the nervous system in anterior view. C: 3D reconstruction of the nervous system in posterior view. D: Cross section at the level of the circummeric nerve ring. E: Posterior view of the rhinophoric ganglion from the cerebropleural ganglion. F: Location of the eye and the statocyst in the cerebropleural ganglion. (B,C: AVIZO 6.4.D,E,F: Micro-CT).

Figura 7. Sistema nervioso de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Reconstrución esquemática do sistema nervioso. **B**: Reconstrución 3D do sistema nervioso en vista anterior. **C**: Reconstrución 3D do sistema nervioso en vista posterior. **D**: Sección transversal a nivel do anel nervioso circumentérico. **E**: Visión posterior do ganglio rinofórico dende o ganglio cerebropleural. **F**: Localización do ollo e do estatocisto no ganglio cerebropleural. (B,C: AVIZO 6.4.D,E,F: Micro-CT).



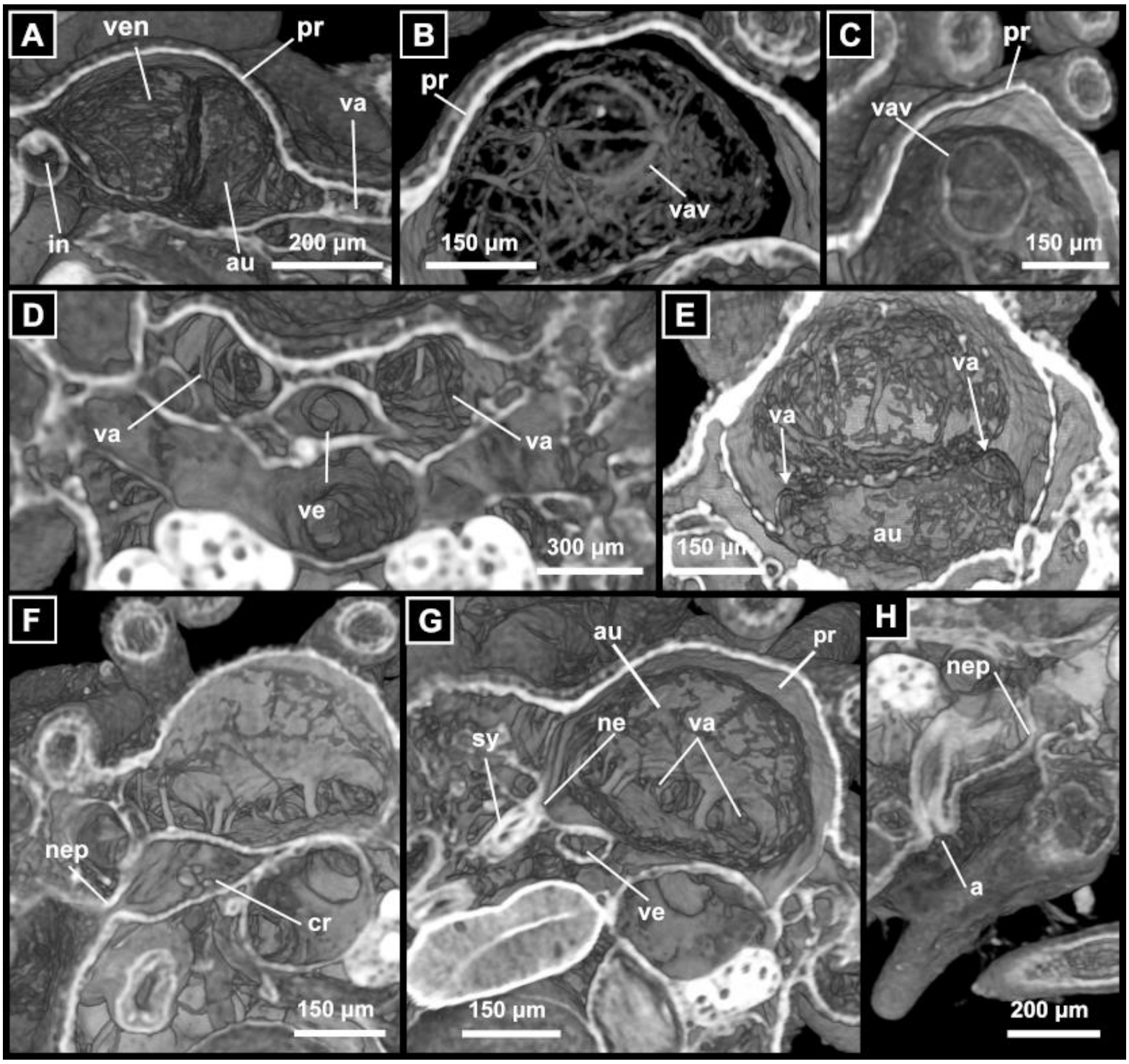
The buccal ganglia are attached to the posterior buccal bulb, located near the pedal ganglia, in the most ventral area of the periesophageal nerve ring. They are noticeably smaller in size and connect to the lower part of the cerebropleural ganglia through a cerebropleural-buccal connective. The buccal ganglia are joined by a short buccal commissure (Figs. 7A,B,C,D).

The rhinophoral ganglia are attached anterodorsally to the cerebropleural ganglia by very short connectives; crosswise they are shaped like a rounded club, but in rear view they are slightly fusiform; a set of nerve bundles emerge from them towards the interior of the rhinophore (Figs. 7A,B,C,D,E).

Renopericardial System: The heart is located under the pericardial bulge that is visible between the second and third pair of ceratal bundles. The heart (Fig. 8A) is surrounded by a pericardium similar in volume and it is bicameral, made up of a posterior auricle and an anterior ventricle, interconnected by an atrioventricular valve (Figs. 8B,C). The circulatory system is open with the hemolymph circulating mainly through sinuses and lacunae. Three main longitudinal vessels stand out in this system, two afferent vessels that open laterally into the auricle (Figs. 8A,D,E) and a medial efferent vessel that runs dorsally to the longitudinal canal of the digestive gland, and opens into the ventricle. (Fig. 8G). Small lateral vessels arise from the efferent and afferent vessels to the left and right, at the level of the lateral ceratal bundles.

Figure 8. Renopericardial system of *Algarvia alba* (Ría de Ferrol - 01110109).A: Heart. B-C: Atrioventricular valve. D: Cross section of the afferent and efferent vessels. E: Opening of the afferent vessels in the auricle. F-G: Renal system and afferent and efferent vessels. H: Location of the anus and nephroproct. (A-H: Micro-CT).

Figura 8. Sistema renopericardico de *Algarvia alba* (Ría de Ferrol - 01110109).**A**: Corazón. **B-C**: Válvula aurículo-ventricular. **D**: Sección transversal dos vasos aferentes e vaso eferente. **E**: Abertura dos vasos aferentes na aurícula. **F-G**: Sistema renal e vasos aferentes e eferente. **H**: Localización do ano e do nefroprocto. (A-H: Micro-CT).



The renal apparatus opens through the nephroproct between the 3rd and 4th ceratal bundles, on the right side of the animal, in the vicinity of the anus (Figs. 4A; 8H). The renopericardial duct connects the renal chamber with the pericardium, where it opens through the nephrostome, where the syrinx is located shortly before the opening (Figs. 8F,G).

Reproductive System: The reproductive system is androdiaulic (Fig. 9A) and can be divided into three distinct regions: hermaphrodite, male, and female. It opens to the outside via the genital atrium, which is located at the base of the second cerata bundle on the right side of the body (Fig. 4D) and is made up of the male genital opening, in the anterior area, and the female genital opening, in the posterior area, both merging into a wide common genital atrium (Figs. 9A,B,G).

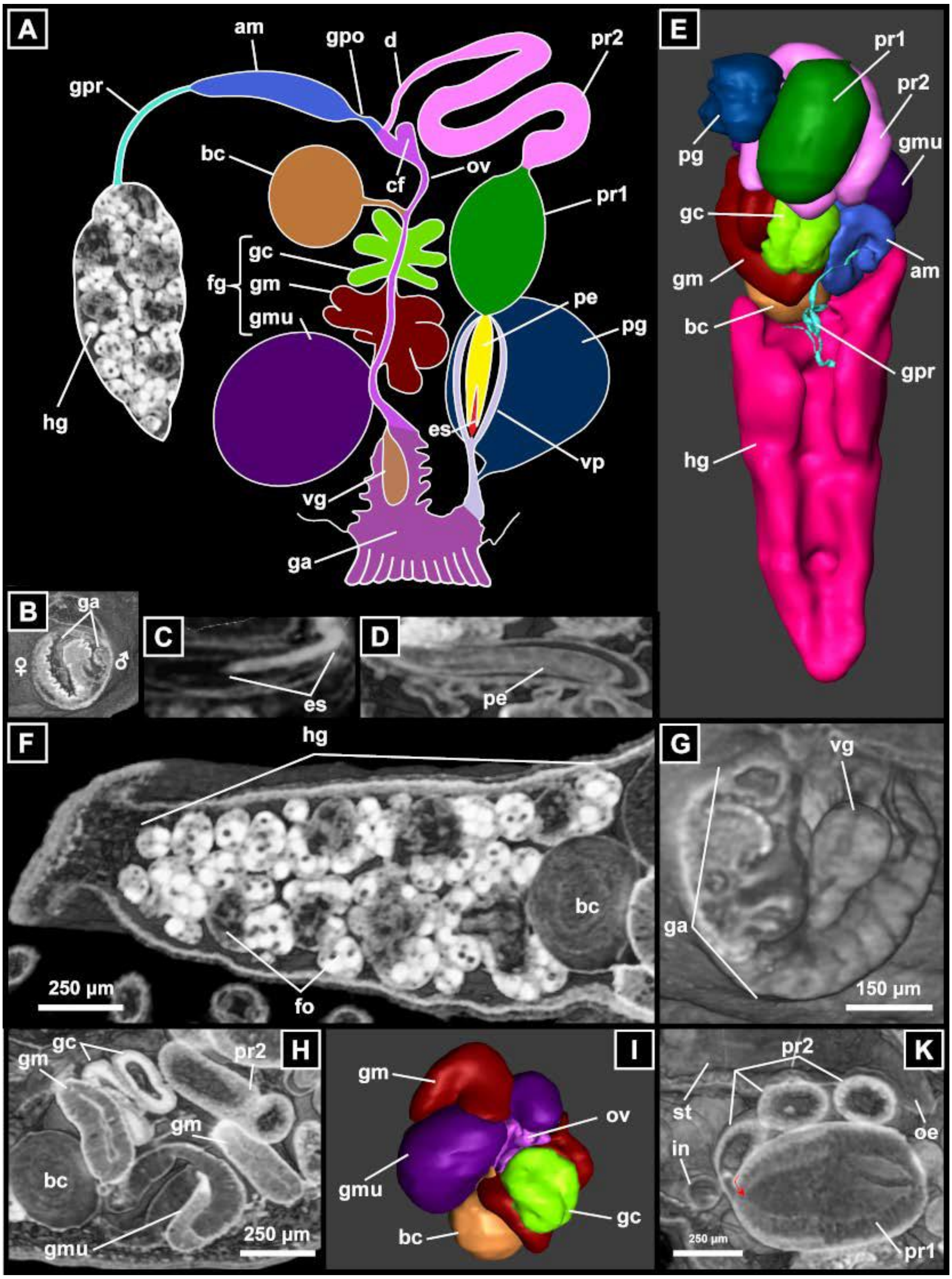
Hermaphroditic reproductive system: The gonad is large and conical, filling almost of the posterior half of the animal. The hermaphrodite gland is made up of a large number of spherical gonadal follicles that give it a clustered appearance (Figs. 9A,E,F). A complex system of gonoducts from the various follicles converge anteriorly into a single preampullary gonoduct, connecting with the elongated ampulla, which is folded upon itself (Figs. 9A,E). Following the ampulla, there is a short postampullary gonoduct that bifurcates into the male gonoduct (vas deferens) and the female gonoduct (oviduct) (Fig. 9A).

Male reproductive system: The vas deferens arises from the postampullary gonoduct, which is prostatic in almost its entire length; it presents a prostate clearly differentiated into two regions, the first region of the prostate is elongated, cylindrical and very contoured, gradually increasing in diameter, from the short vas deferens to the end, where it connects with the second prostatic region, which is very broad, globose and oval, finally connecting with the penis (Figs. 9A,E,I,K).

The penis is large, cylindrical, elongated and is provided with a penial stylet, which when retracted is housed in a penial sheath and the entire complex is practically surrounded by a large penial gland (Figs. 9A,C,D).

Figure 9. Reproductive system of *Algarvia alba* (Ría de Ferrol - 01110109). A: Schematic reconstruction. B: Genital atrium. C: Penial stylet, D: Penis and penial sheath. E: 3D reconstruction of the reproductive system. F: Copulatrix bursa and gonadal follicles in a frontal section at the mid-height of the hermaphrodite gland. G: Vestibular gland in the genital atrium. H: Transverse section of the female gland and bursa copulatrix. I: 3D reconstruction of the female gland and bursa copulatrix. K: Cross section of the two regions of the prostate. (E,I: AVIZO 6.4.; B,C,D,F,G,H,K: Micro-CT).

Figura 9. Aparato reprodutor de *Algarvia alba* (Ría de Ferrol - 01110109). **A**: Reconstrución esquemática. **B**: Atrio xenital. **C**: Estilete peneal, **D**: Pene e vaina peneal. **E**: Reconstrución en 3D do aparato reprodutor. **F**: Bulsa copuladora e folículos gonadais nunha sección frontal á altura media da glándula hermafrodita. **G**: Glándula vestibular no atrio xenital. **H**: Sección transversal da glándula feminina e bulsa copuladora. **I**: Reconstrución en 3D da glándula feminina e bulsa copuladora. **K**: Sección transversal das dúas rexións da próstata. (E,I: AVIZO 6.4.; B,C,D,F,G,H,K: Micro-CT).



Female reproductive system: The proximal part of the oviduct has a widening of the lumen that forms the fertilization chamber (Fig. 9A). In front of this chamber, the duct of the bursa copulatrix opens into the oviduct, which is spherical in shape, large in size, and located ventrally in front of the hermaphrodite gland (Figs. 9A,E,F,H,I). During its course towards the outside of the reproductive system, the oviduct receives the ducts from the female glands, in this order: capsule gland, membrane gland, and mucus gland, the latter being oval and larger than the other two and located in ventral position (Figs. 9A,E,H,I). In the final section of the oviduct, a vestibular gland appears externally in the female genital atrium, occupying most of the opening of the female genital atrium (Figs. 9A,G).

Habitat and Feeding behavior: Little is known about the habitat of this species. García-Gómez & Cervera (1989) only specified that they were collected at the entrance of the port, on a bottom with rocks and silt at a depth of 13 m. However, Silva *et al.* (2016) indicated that the photograph of the specimen of *Algarvia alba* was taken at a similar (14 m), in an area of sand and flat stones; this habitat closely agrees with that of the specimen from the Ría de Ferrol collected on a medium sand bottom with some flat rocky outcrops at 20 m depth, which seems to suggest a preference of this species for sandy substrates with the presence of rocks or flat stones.

The diet of *Algarvia alba* is unknown, but our specimen was observed moving actively with a clearly protruding buccal area in an apparently aggressive attitude (Figs 2A,D,F), similar to *Aeolidia papillosa* when it approaches the anemones it preys upon. The actiniarian *Sagartia undata* (Müller, 1778) is very common in the habitat where *Algarvia alba* was collected in the Ría de Ferrol and therefore it is likely that might constitute part of its food source.

DISCUSSION

The original description of *Algarvia alba* by García-Gómez & Cervera (1989) was based on two specimens collected in Sagres (Algarve, southern Portugal). The only specimen collected after then, corresponds to the one from the Ría de Ferrol described herein. As mentioned above, the second record of *Algarvia alba* is of a specimen photographed from Getaria (Gipuzkoa), (Silva *et al.*, 2016).

The description by García-Gómez & Cervera (1989) generally matches the data presented herein, although most of the results of the present study are novel, considerably broadening the knowledge of the internal anatomy of *Algarvia alba*. The original description of *Algarvia alba* by García-Gómez & Cervera (1989), reports a total of 8 pairs of ceratal bundles, while in the specimen studied here there were 9 pairs. In addition, the ceratal formula of the largest individual found on the Algarve coast was I-5, II-4, III-3.4, IV-3, V-3, VI-3, VII-2, VIII-1 -2, which differs from the ceratal formula of the specimen from the Ría de Ferrol: I- 8, II-6, III-3, IV-5, V-4, VI-4, VII-4, VIII-3, IX- 3 on the left side and I-8, II-6, III-5, IV-5, V-5, VI-5, VII-5, VIII-5, IX-2 on the right side. Furthetmore the 5-mm specimen from Sagres had a total of 50 cerata while that from Ría de Ferrol has 86 cerata. Thus, it appears that this species presents a great variability in the number of cerata, since the two specimens had the same length. Moreover, the size of specimen photographed in Getaria was estimated between 5 and 10 mm and showed a ceratal number very similar to our specimen (Silva et al., 2016).

Regarding the position of the natural orifices in the animal, there are several discrepancies between the original description and the specimens studied here with Micro-CT. Thus, García-Gómez & Cervera (1989) affirmed that "the anus is cleioproctic, located behind the lower ceras of the first postpericardial row" and that "the nephroproct is situated between the second and third ceratal bunles, within the interhepatic space”. However the nephroproct and the anus are actually postpericardial, located between the 3rd and 4th right ceratal bundles (Figs. 4A; 8H). García-Gómez & Cervera (1989) also indicated that "the gonopore [*sic.*] is also located within the interhepatic space on the right side of the body, at the pericardial level" In fact, the genital atrium of our specimen is not interhepatic clearly and it is located just below the second right ceratal bundle (Figs. 4A-D); the posterior border of the genital atrium corresponds to the cross section of the exit of the intestine from the stomach that is anterior to the frontal edge of the ventricle (Fig. 8A).

On the other hand, the coloration and the shape of the oral tentacles, rhinophores, and cerata match those in the original description very closely, with some exceptions. For instance, García-Gómez & Cervera (1989) stated that "the rhinophores have 5 oblique lamellae arched posteriorly", but these are not really lamellae, rather they are nine knot-like thickenings with a semicircular section. These thickenings are arranged obliquely from the outer to the inner edge and, as described, the five lower thickenings are oblique and the four upper ones are transversely circular (Figs. 3B,C; 2C). Additionally, García-Gómez & Cervera (1989) neither described the knotted edge of the velar sides behind the oral tentacles, nor those on the front edge between the tentacles (Fig. 2E), and neither García-Gómez & Cervera (1989) nor Silva *et al.* (2016) mentioned the presence of eyes, which are clearly evident on a lateral view; in SILVA *et al.* (2016, fig. 1) the left eye of the animal is perfectly visible. Finally, García-Gómez & Cervera (1989) did not mention the presence of statocysts, oral glands or salivary glands that are present in the specimen from the Ría de Ferrol.

García-Gómez & Cervera (1989) only described the radula and jaws in the digestive system. According to these authors, the radula ribbon had a total of 23 teeth, while the specimen from the Ría de Ferrol, with a similar size, had 29 teeth, of which 19 were on the superior portion and 10 in inferior portion, but the teeth morphology is the same in both radulae. The rest of the anatomy of the digestive system of *Algarvia alba* studied herein has never been described before, as well as the anatomy of the nervous, circulatory, and excretory systems.

The reproductive system of the specimen studied is androdiaulic, which according to Ghiselin (1966) is made up of two separate genetical ducts. Ghiselin (1966) also indicated that the androdiaulic condition is not typical in aeolid nudibranchs, in which triaulic condition predominates. According to (Wägele & Willan, 2000) a triaulic reproductive system is a synapomorphic characteristic of nudibranchs, but some exceptions have been reported. García-Gómez & Cervera (1989) provided an incomplete description of the reproductive system. For example, in the male reproductive system they do not mention the existence of a penial gland, which, as has been verified in our specimen, surround the copulatory organ. Furthermore, García-Gómez & Cervera (1989) mentioned the existence of a simple and unarmed penis, while the specimen studied here has a clear penial stylet at the end of the penis. Although García-Gómez & Cervera (1989) described a spherical prostate that joins the penis, they did not recognize a second prostatic region that they identified as a long vas deferens. Finally, García-Gómez & Cervera (1989) do not mention the existence of a vestibular gland that is located at the exit of the oviduct in the genital atrium.

García-Gómez & Cervera (1989) only described the following from the female reproductive system of *Algarvia alba*: “The location of the seminal receptacle was not observed, probably due to its internal position within the female gland and also to the small size of the specimen”, thus presupposing the existence of a seminal receptacle without seeing it. This assertion is possibly based on Ghiselin (1966) that stated that aeolid nudibranchs lack a bursa copulatrix. However, the specimen studied here has a large, spherical bursa copulatrix located ventrally in front of the hermaphrodite gland, therefore, not located internally in the female gland. The present study describes for the first time the shape and distribution of the glands that make up the female gland: capsule gland, membrane gland, and mucus gland, and confirms that there is no seminal receptacle.

Due to the absence of molecular data, the phylogenetic position of the genus *Algarvia* remains uncertain. According to García-Gómez & Cervera (1989), the genus *Algavia* was morphologically characterized by the presence of a cleioproctic anus, an interhepatic nephroproctus, and a uniseriate radula with denticulate teeth. García-Gómez & Cervera (1989) argued that these characters are consistent with the definition of the family Facelinidae according to the definition of Gosliner (1979), and therefore they included *Algavia* in this group. More recently several molecular studies have indicated that the family Facelinidae is paraphyletic (e.g., Carmona *et al.* 2015; Goodheart *et al.*, 2017; Martynov *et al.* 2019) resulting in the separation of the family Myrrhinidae Bergh, 1905 as proposed by Martynov *et al.* (2019). However, Facelinidae and Myrrhinidae are not well defined from a morphological point of view, for example, the genus *Myja* is considered a member of Facelinidae despite having an acleioproctic anus (Martynov *et al.* 2019). At the moment, it is not possible to determine the taxonomic position of *Algavia* until there is an in-depth review of the systematics of the different families, including molecular phylogenies and anatomical descriptions. For these reasons, in the present study we propose to maintain the genus *Algarvia*, as a member of Facelinidae *sensu stricto* until more information becomes available.

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