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New shallow-water nudibranch records (Mollusca: Gastropoda: Heterobranchia) from North West Atlantic coast of Iberian Peninsula

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Abstract: Samplings carried out in the last years by the "Grupo de Estudo do Medio Mariño" (GEMM) in Ría de Arousa, have resulted in the discovery of four species never recorded before in the Iberian Peninsula waters. New data on the feeding habits of *Atagema gibba* Pruvot-Fol, 1951, *Onchidoris bilamellata* (Linné, 1767) and *Armina neapolitana* (Delle Chiaje, 1824) are given, as well as details about the number of specimens observed and reproductive behavior, which leads us to assume the presence of stable populations of these species in the area. *Flabellina affinis* (Gmelin, 1791) was previously reported for Galician waters, but according to the published image, this seems to have been misidentified with *F. ischitana* Hirano & Thompson, 1990. We present here new data about the presence of large numbers of this southern species in the area.

Résumé : *Nouveaux signalements de nudibranches (Mollusca : Gastropoda : Heterobranchia) des côtes nord-ouest atlantiques de la péninsule ibérique.* Les échantillonnages effectués ces dernières années par le "Grupo de Estudo do Medio Mariño" (GEMM) à Ría de Arousa ont permis de découvrir quatre espèces jamais citées précédemment dans les eaux de la péninsule ibérique. De nouvelles informations sur les habitudes alimentaires d'*Atagema gibba* Pruvot-Fol, 1951, *Onchidoris bilamellata* (Linné, 1767) et *Armina neapolitana* (Delle Chiaje, 1824) sont données, ainsi que quelques détails sur le nombre de spécimens observés et le comportement reproducteur, qui nous amènent à supposer la présence de populations stables de ces espèces dans la région. *Flabellina affinis* (Gmelin, 1791) a déjà été signalée pour les eaux galiciennes mais, d'après l'image publiée, elle semble avoir été confondue avec *F. ischitana* Hirano & Thompson, 1990. Nous présentons ici de nouvelles données sur la présence d'un grand nombre d'individus de cette espèce méridionale dans la région.

Keywords: Mollusca ● Opisthobranchia ● Nudibranchia ● Atlantic ● Iberian Peninsula

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Introduction

Nudibranchia is a well-known taxon, with more than 3000 described species worldwide (Wägele et al., 2005) in part due to their bright colors and shapes, which make the observations on this group more frequent and not only limited to sample official programs (Richling, 2014), especially those inhabiting shallow waters. Despite this, studies devoted to the nudibranch fauna of the Spanish coasts were scarce until well into the second half of the twentieth century (Ballesteros, 2007). This situation has changed in the last decades, during which the publication of numerous studies on the group has attained a level of knowledge of the Spanish nudibranch fauna that is among the most comprehensive in Europe (Cervera et al., 2006; Urgorri et al., 2011).

The Galician region, with more than 1500 km of coastline, and highly productive waters due to the seasonal upwelling phenomenon (Alvarez et al., 2012), presents a great diversity of habitat and species. Currently, approximately a hundred nudibranch species have been reported from this area (Urgorri & Besteiro, 1983; Urgorri et al., 2011), although this number has continued to increase since the last inventory was released, with the publication of new records of species outside its known distribution range (Díaz-Agras et al., 2010; Almón et al., 2013) and even description of new ones (Caballer et al., 2016).

However, not all the genera in the order Nudibranchia have been studied to the same extent, mainly due to the difficulties derived from elusive habits displayed by certain species, small populations or size and cryptic color patterns (Hunnam & Brown, 1975; Akyol & Saglam, 2014), being in many cases difficult to locate and collect.

In this study we present new data about species belonging to four different genera (*Atagema* Gray, 1850; *Onchidoris* Blainville, 1816; *Flabellina* Gray, 1833 and *Armina* Rafinesque, 1814), that display some of these characteristics, recently found along Galician coasts for the first time, during the surveys carried out by the non-profit-making organization "Grupo de Estudo do Medio Mariño" (GEMM) within the remit of its project to catalog the marine fauna of the coasts of Galicia.

The genus *Atagema* has only two representatives in European waters, *Atagema gibba* Pruvot-Fol, 1951 and *A. rugosa* Pruvot-Fol, 1951 (Costello et al., 2008). *A. gibba* is considered very rare owing to the fact that it has been reported only in a few occasions since its description in 1951 from the French Mediterranean. Two of this records are from the southwest of England and the other are unpublished records in Italy and France (Rudman, 2001a; Thompson & Brown, 1974). The available information on the species is therefore very scarce.

There are up to thirteen different species within the genus *Onchidoris* in European waters (Costello et al., 2008), nine of them being recorded along the Spanish coasts so far (Cervera et al., 2006). These are small oval species, generally covered by a spiculose mantle dorsally with rounded tubercles (Alvim et al., 2011). Most of the species of this genus are bryozoan feeders, living camouflaged among their prey (Ortea & Ballesteros, 1982). The Family Arminidae are poorly known nudibranchs, in part due to the fact that they burrow in the sediment and that they live mainly in deep water (Kolb, 1998). Five valid species of the genus *Armina* were recorded in European waters, four of which has been reported within the Iberian Peninsula so far. Three of them have Mediterranean and adjacent Atlantic distribution (*A. maculata* Rafinesque, 1814, *A. neapolitana* (Delle Chiaje, 1824) and *A. tigrina* Rafinesque, 1814) (Ballesteros et al., 2012), and *A. loveni* (Bergh, 1866) is known for the north Atlantic, with its southern limit along the French Atlantic coast, with some records showing traces along Portuguese coasts and Canary Islands (Cervera et al., 2006).

Lastly, the genus *Flabellina* is made up of 20 different species within European waters (Caballer et al., 2014), with five species reported for Galician waters (Cervera et al., 2006).

The aim of this study is therefore to inform about the presence of these species in Galician waters for the first time and provide some new information about their biological make-up.

Materials and Methods

Material examined for this study came from Grupo de Estudo do Medio Mariño (GEMM) continuous SCUBA research program, devoted to the improvement of the knowledge about the marine biodiversity of Galician waters. All the specimens were observed during a series of diving surveys carried out since 2010 for the GEMM group along the Galician coast. Samples included in this study where obtained between 2013 and 2016, in different localities of the Ría de Arousa and the Illas Atlánticas Maritime National Park, by scuba diving (Fig 1).

In this area, the maximum depth ranges from 40 to 60 meters which makes most of the area accessible to sampling by scuba diving. Morphological observations of external features as well as notes on coloration were taken from live specimens and then photographed *in situ* showing the habitat where they were found. Measurements were taken from live specimens.

After a preliminary identification, two individuals of *A. gibba*, two of *A. neapolitana* and eight of *Onchidoris bilamellata*, were taken to the laboratory for morphological examination under the stereomicroscope. These specimens were then preserved in 98% ethanol, after being anesthetized by partial freezing so as to prevent bodily contraction. No further studies were conducted for *F.*

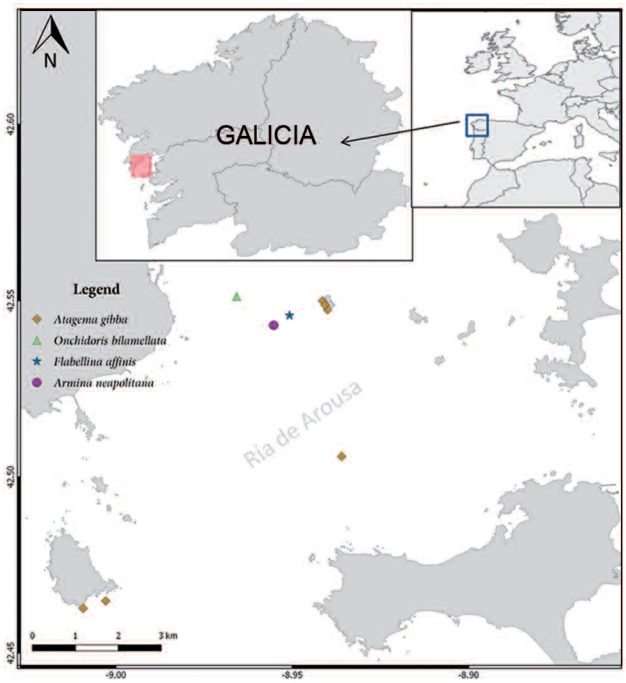

Figure 1. Map showing the observation localities of four species studied.

affinis, since collection of specimens was not possible at the time of observation. One of the specimens of *A. gibba* was employed for radular studies by using a Quanta 200 Scanning Electron Microscope (SEM), in order to complete morphological data. SEM samples were prepared by partially dissolving buccal masses in 10% NaOH solution, washed, air dried, then mounted on aluminum stubs without coating treatment. Due to the limited access to SEM facilities, no other radular studies were performed. Sponge samples were sent to Dr. Manuel Mandonado, from the Sponge Ecobiology and Biotechnology Group at the Department of Marine Ecology in the Centro de Estudios Avanzados de Blanes (CEAB) for identification.

The preserved specimens were deposited at the Museo de Historia Natural da Universidade de Santiago de Compostela (MHNUSC, Santiago de Compostela, Spain) under accession number (pending manuscript acceptance).

Systematic descriptions

Phyllum MOLLUSCA
Class Gastropoda
Order Nudibranchia
Family Discodorididae Bergh, 1891
Genus *Atagema* Gray, 1850
Atagema gibba Pruvot-Fol, 1951
(Fig. 2)

Material examined

42.4627N-09.0093W, Sálvora Island (Punta Pirula) 1 specimen, spawning, 18 m depth, 18 August 2013 (MHNUSC 25127); 42.4648N-09.0029W, Sálvora Island (Punta Brisan), 1 specimen, spawning, 18 m depth, 18 August 2013; *Specimens observed:* 42.5058N-08.9360W, "Os Esqueiros", 1 specimen, 20 m depth, 28 June 2014; 42.5474N-08.9400W, Rúa Island, 2 specimens, one of them spawning, 8 m depth, 2 July 2014; 42.5488N-08.8407W, Rúa Island, 10 specimens, four of them spawning, 15 m depth, 22 July 2014; 42.5499N-08.8414W, Rúa Island, 4 specimens and several spawn masses, 10 m depth, 12 August 2014; 42.5488N-08.9407W, Rúa Island, 1 specimen spawning, 15 m depth, 4 September 2014.

Description

In general, our specimens agree with the description given by Thompson & Brown (1974). Living animals between 40-50 mm length, 36 mm when preserved. General aspect of the body rather flattened, but with a characteristic "zig-zag" narrow ridge down the dorsal midline, between the bases of rhinophores to the gills. Mantle covered with small digitiform whitish spiculose tubercles, forming a white net-like pattern over a darker brownish-orange background. Mantle color quite variable, more frequently light-brown or orange (Fig. 2A & B).

<u>*Radula*</u>. Teeth are smooth with an erect hook-like cusp placed in rows where the size is reduced near the mid-line and near the periphery. Radular formula 29/30-0-29/30 (Fig. 2C & D).

Biology

Due to the fact that there is no previous information about prey selection, beyond that which feed on sponges (Picton & Morrow, 2016), data about prey selection was gathered. When a specimen was located over a sponge mass, samples of the sponge were then collected and kept in absolute alcohol for subsequent identification, resulting in all cases in the species being *Haliclona cinerea* (Grant, 1826), which, as with other species of this sponge genus, is highly polymorphic and polychromic (more often purple, yellow, and white) (Fig. 2E). This polymorphic pattern also occurs in *A. gibba* and is presumably associated with the morphotype of porifera which they feed on. Albino specimens were found on white colored *H. cinerea,* while violet brown ones were found on the purple forms of the same sponge. The ability of the nudibranch to assume the color and shape of the sponge which it feeds on seems to allow the nudibranch to remain unnoticed.

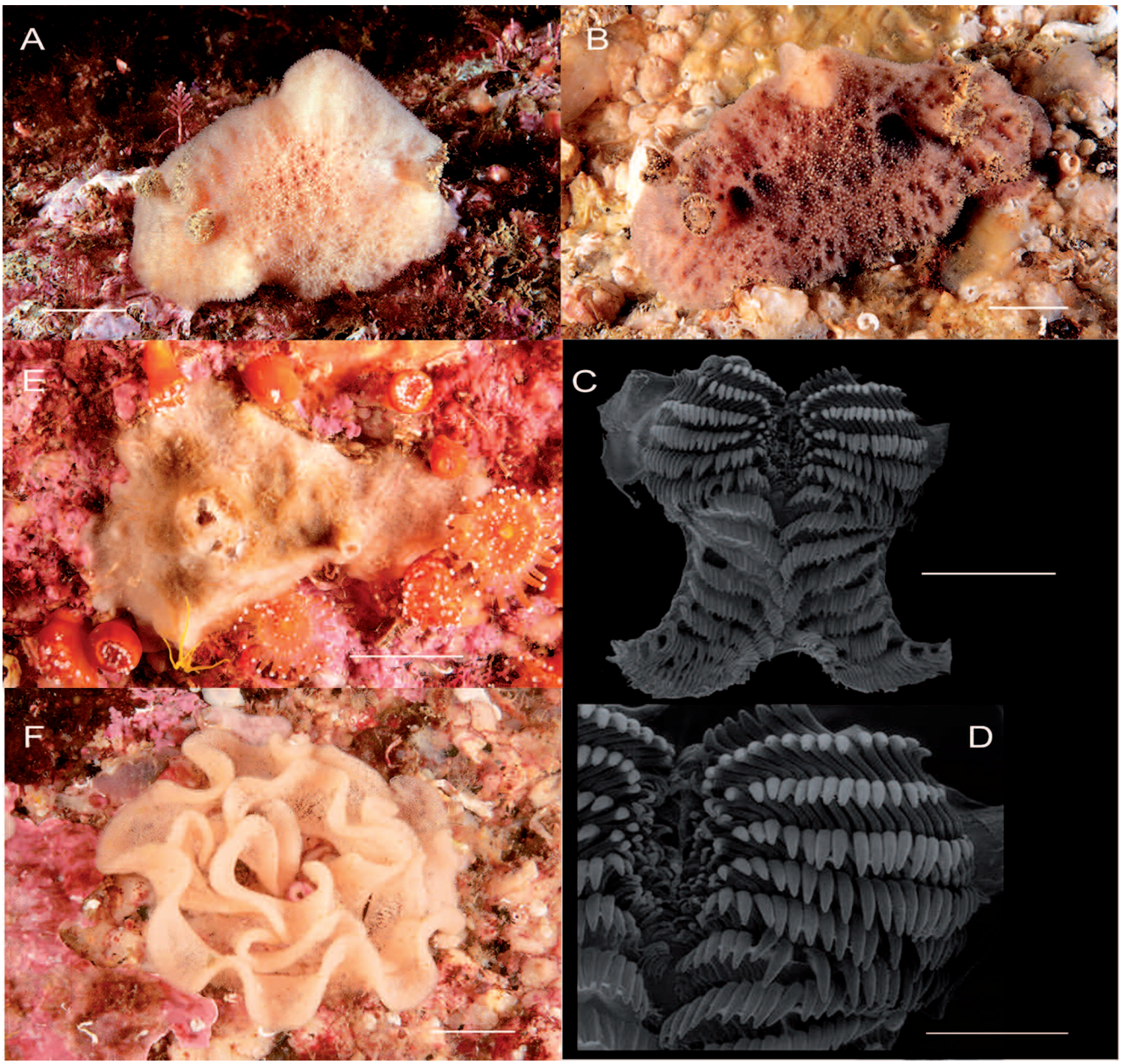

Figure 2. *Atagema gibba.* **A-B.** Macro shots of different morphotypes. **C-D.** SEM photography of the radula. **E.** Sponge *Haliclona cinerea*. **F.** Egg ribbon. Scale bars: **A** = 10 mm, **B** = 10 mm, **C** = 1 mm, **D** = 0.5 mm, **E** = 20 mm, **F** =10 mm.

Remarks

A. gibba can be distinghished from *A. rugosa* by several key characters. *A. gibba* is larger, with a crenulated mantle edge, a coarsely granulated mantle, and five lobes around the branchial sheath that are unequal in size, being the dorsal ones, larger and almost horizontal, partial hiding the gills (that are also unequal and posteriorly directed) (Thompson & Brown, 1974). The dorsal ridge is also present in *A. rugosa*, but is much smaller, and it has no prebranchial bump at the end of the ridge which is very conspicuous in *A. gibba*. *A. rugosa* has also a white background with large black spots organized into two longitudinal lines on each side of the dorsum (Gosliner, 1987), while *A. gibba* is usually brown and shows black spots alongside the prebranchial bump, placed in a recessed portion of the mantle (Camacho-García & Gosliner, 2008).

Spawning masses were found in Galician waters only within July and August, although spawning masses were observed in September on higher latitudes (Rudman, 2001a). Differences in spawning season may be due to water temperature variation, although there is no data from

other localities. Temperature in Galician localities on the days when the specimens were found ranged from 16 to 19°C at surface. Temperature data were obtained from the Regional Model Oceanographic Spanish Institute of Oceanography (www.indicedeafloramiento.ieo.es).

Spawning coil was previously reported to consist of a ruffled yellow ribbon of eggs deposited in a spiral of around 11/2 turns (Picton & Morrow, 2016), even though we found some with up to 30/3 turns (Fig. 2F).

After more than ten years monitoring marine species in the Ría de Arousa, the presence of this species has never been reported before, so the presence of a total of nineteen specimens within a year is surprising, although it must be taken into account that its great camouflage capability can easily make it go unnoticed. This is the first record of this species for the Iberian Peninsula, and the southernmost record for the species.

Family Onchidorididae Gray, 1827
Genus *Onchidoris* Blainville, 1816
Onchidoris bilamellata (Linnaeus, 1767)
(Fig. 3)

Material examined

42.5512N-08.9658W, "O Petón Baixo", 8 specimens spawning, under stones, 22 m depth, 04 March 2017 (MHNUSC 25125).

Specimens observed

42.5512N-08.9658W, "O Petón Baixo", Group of at least 30 specimens during massive spawning, 18 m depth, 19 July 2014.

Description

Living specimens were 8-12 mm length. The body is a mottled mixture of brown and creamy white with lighter papillae, which are larger in the periphery decreasing in size and less densely spaced towards the dorsal midline. Rhinophores are long and narrow, with 15-20 leaflets on either side, arising from sockets without raised sheaths. Up to 32 unipinnate gills are arranged in a horseshoe around the anal papilla. The gills are completely contractible into separate pockets beneath the mantle. The gill circlet is surrounded by tubercles (Fig. 3A).

Biology

A group of at least 30 small individuals was located in a rocky bottom, in a small cave between rocks. They were laying spawning masses under a stone covered by *Verruca stroemia* (Müller, 1776) (Fig. 3B).

Remarks

Several key characters permit to distinguish *O. bilamellata* from the other European species. The size is very useful when dealing with adults, since *O. bilamellata* is the largest species within this genus in Europe. The color pattern is also a clear character to distinguish it from the other species.

The horseshoe shape of the gill circlet is shared only by *O. muricata* (O.F. Müller, 1776). *O. muricata* has a close general appearance, but is smaller, with a horseshoe gill pattern not flattened as in *O. bilamellata*, and with flat-top tubercles in the mantle with spicules arising throw the apex (Chichvarkhin et al., 2016).

The rhinophores are longer in *O. bilamellata* than in other species and bear up to 16 lamellae. Only *O. tridactila*

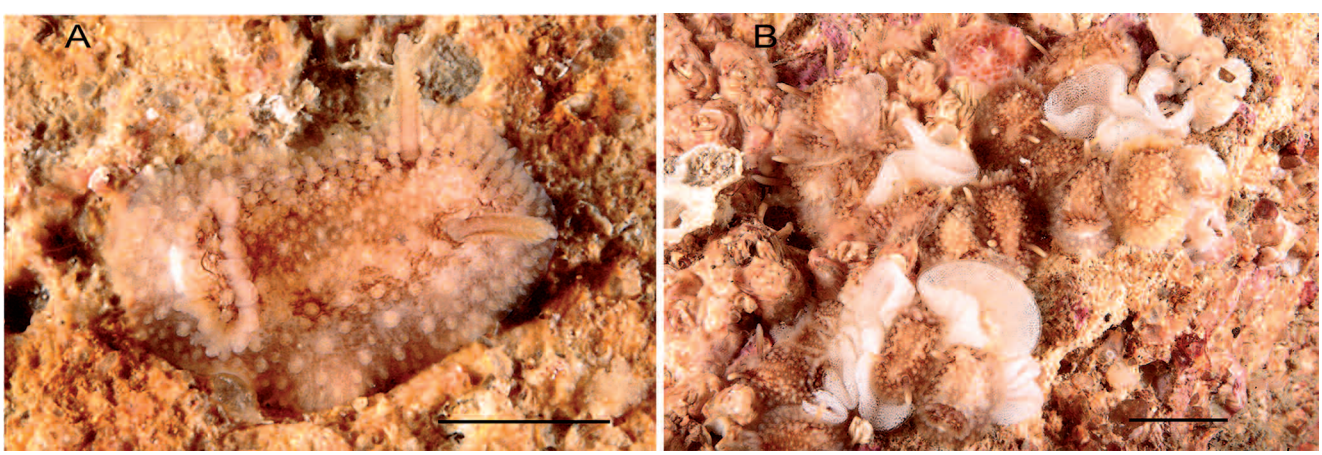

Figure 3. *Onchidoris bilamellata.* **A.** Macro shot of a single specimen. **B.** Group of individuals during a massive spawning event. Scale bars: **A** = 5 mm, **B** = 10 mm.

Ortea & Ballesteros, 1982, has a similar number of lamellae, but it is clearly distinguished by its coloration, whitish with orange spots aligned along the dorsum (Ortea & Ballesteros, 1982; Alvim et al., 2011; Ortea et al., 2014).

Aggregations of a large number of individuals of these species already spawning have been known since the beginning of the 20th century (Claverie & Kamenos, 2008) and have been subsequently cited on several occasions (Rudman, 2001b). In the North of the United Kingdom it has been observed that the spawn masses in *O. bilamellata* occurs between December and April (Claverie & Kamenos, 2008), but that sometimes, there may be an additional period in summer due to favorable environmental conditions in which individuals have a precocious maturity. This appears to be the case of the specimens observed in the Ría de Arousa.

This species is considered to have an annual life-cycle (Bleakney & Saunders, 1933). Different explanations were posited to explain the disappearance of the individuals after spawning, including massive after-spawning dead, littoral and subtidal migrations or colonization from other localities (Bleakney & Saunders, 1933; Todd, 1979 & 1985). The most recent experiments support the spawning migration movements theory, moving from their natural intertidal distribution areas to subtidal locations, returning back into shallower water (Claverie & Kamenos, 2008). We have no previous records of *Onchidoris bilamellata* in the intertidal or subtidal areas from Galician waters, so in this case we would surmise that they had arrived from other distant areas.

In addition to this, literature about feeding habits of *O. bilamellata* describes this nudibranch as a predator of the barnacle *Semibalanus balanoides* (Linné, 1767) (Todd, 1979; Claverie & Kametos, 2008), and that the early planktonic stages of the latter induces the colonization of the first. In the Ría de Arousa *O. bilamellata* were found on rock surfaces covered with the barnacle *Verruca stroemia* (O.F. Müller, 1776). The fact that *O. bilamellata* may prey on other barnacles, including *V. stroemia*, has been previously pointed out (Todd, 1979) but it was considered only viable for adults, the juveniles being incapable of handling this species. In Galicia, it is also possible to find *S. balanoides*, but *O. billamellata* had never been found before, which would support the theory of a settlement from distant areas. Future research should clarify whether this species has been successfully established, using feeding areas with *S. balanoides,* or whether they follow a different strategy. This is the first record of this species for the Iberian Peninsula, and the southernmost record for the species.

Family Flabellinidae Berg, 1889
Genus *Flabellina* Gray, 1833
Flabellina affinis (Gmelin, 1791)
(Fig. 4)

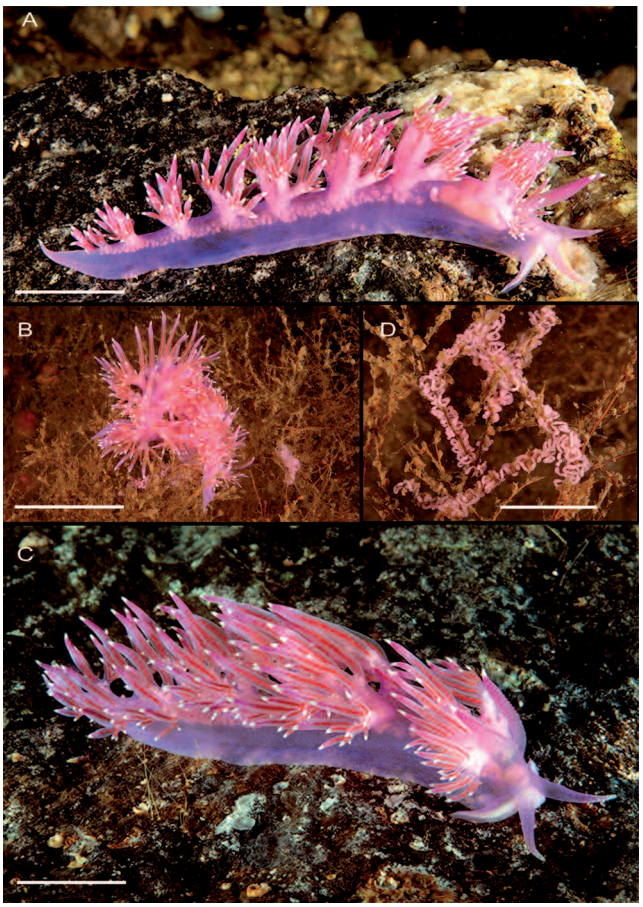

Figure 4. *Flabellina affinis.* **A, C.** Detailed photography of single mature specimen. **B.** Two individuals mating. **D.** Egg masses. Scale bars: **A** = 10 mm, **B** = 40 mm, **C** = 10 mm, **D** = 30 mm.

Specimens observed

42.5458N-8.9508W, "Torre de Abajo", massive spawning masses of more than 100 individuals, 24-32 m depth, throughout the month of July 2015.

Description

During the month of July, hundreds of individuals were observed together within a relative small area. The mature specimens of different size reached 50 mm (Fig. 4A-C), some were still mating (Fig. 4B) but most were already laying their spawning masses (Fig. 4D).

Biology

Factors that have led to this sudden appearance of high number of individuals of a species never recorded before in this area remain unknown.

Remarks

In the Iberian Peninsula, this species is well-known both in

Mediterranean and adjacent Atlantic waters (Cervera et al., 2006). There were no previous records of the species in Galician coasts until 2011 (Urgorri et al., 2011). However, the photograph included in this work seems to belong to a different species, based mainly on visible morphological characters, such as general color pattern and space occupied by digestive glands inside the cerata. Although not definitive, the presence of a dark pinkish patch just beneath the white cnidosacs is considered characteristic of *F. affinis* (Schulze & Wägele, 1998). We believe the species included in that work is more likely to be *F. ischitana* Hirano & Thompson, 1990. Both species share some morphological characteristics that make their separation more challenging, but given the impossibility of examining the specimens in more detail, we have based our opinion on the visible and widely-accepted morphological characters.

Family Arminidae Iredale & O'Donoghue, 1923 (1841)
Genus *Armina* Rafinesque, 1814
Armina neapolitana (Delle Chiaje, 1824)
(Fig. 5)

Material examined

42.543N-8.9553W, "O Avispón" 2 specimens spawning, 34 m depth, 14 November 2015 (MHNUSC 25126).

Specimens observed

Massive spawning group of more than 1000 individuals of this species along with a high number of *A. maculata*.

Description

Living specimens of *A. neapoliotana* reaching 42 mm length, 20-22 mm when preserved. Body elongated, flattened and narrowing towards the posterior. Living animals reddish light brown with a well-marked white longitudinal line extending all along the body, between the rhinophores and the tail. Margins of the mantle also white. Between the central line and the borders there are several less pronounced longitudinal white lines. One conspicuous transversal thicker white stripe in the midline of the body, which intersects the longitudinal center line. Some dark-blue patches in frontal margin of the body, near the tail and all along the foot. Two club-shaped rhinophores are present, with 9 to 11 vertical lamellae each (Fig. 5A).

Biology

A vast number of individuals of both *A. neapolitana* and *A. maculata* species were found occupying the muddy bottoms all around a rocky protrusion in its entirety. These bottoms are mainly covered by *Veretillum cynomorium* (Pallas, 1766) which *Armina* species feeds on. Spawning masses of both species laid next to each other without separation between species (Fig. 5B). The eggs within a narrow gelatinous ribbon, forming an elongated irregular mass, with numerous and very tight turns. Shape similar in both species, been those of *A. maculata* much larger (16 × 6 cm) than in *A. neapolitana* (4 × 1.5 cm).

Remarks

Armina neapolitana was described based on a specimen from French Brittany under the name of *Pleurophyllidia vasconica* (Delle Chiaje, 1824). Externally, *A. neapolitana* can be distinguished from other European species by its size and color pattern. *A. maculata*, has no notal ridges but irregulary arranged white pustules on the orange colored notum. In *A. loveni*, the notum is brick-red, with up to 50 prominent white longitudinal wrinkles, while in *A. neapolitana* are less numerous (24-25) and with a conspicuous central thick white band. In *A. tigrina*, the longitudinal ridges (up to 29) are surrounded by black pigment, giving a much darker, blackish appearance to the mantle (Kolb, 1998). *A. tricuspidata*, has a dark-grey mantle with a pattern of low white pustules, very far from the appearance of *A. neapolitana* (Thompson et al., 1990).

Previous reports about *Armina* species in the Iberian

Figure 5. *Armina neapolitana*. **A.** Living specimen. **B.** Egg masses of *A. maculata* (frontmost) and *A. neapolitana* (background). Scale bars: A = 10 mm, B = 40 mm.

Peninsula are scarce and sparse, due to the difficulties derived from their living habits (Ballesteros et al., 2012; Cervera et al., 2006). Notwithstanding the fact that in recent decades some good descriptions of the morphology and anatomy of several species have been made (Kolb, 1998; Akyol & Saglam, 2014), there are still many gaps regarding their biology and ecology behavior. This represents the northernmost record of *A. neapolitana* in Atlantic Iberian waters (the previous one located on Portuguese coasts, north of Lisbon), and reports a massive reproductive event that involves both species.

Conclusions

In spite of the great work accomplished by Cervera et al. (2006) updating most of the available data of nudibranch fauna of Spain and Portugal, there is still much unpublished information that can contribute to complete the knowledge about this group in the area. Moreover, beyond the remarks on the presence of certain species in our waters, it is of paramount importance to continue filling in the gaps about their biology, color pattern of living specimens as well as basic aspects, such as their diet or reproductive behavior.

Variations in the distribution of species are in many cases related with larval dispersion dynamics, process that can be limited or facilitated by temperature gradients and ocean currents (Wares et al., 2001). Owing to continuous changes in environmental conditions occurring throughout the last decades (Philipart et al., 2011), it is expected that the faunal composition will keep changing as well. Many species have been found to extend their distribution range limits northwards, mainly due to the general rise in water temperature (Philipart et al., 2011) combined with the increasing numbers of species being transported outside their natural area (Almón et al., 2013; Cuesta et al., 2016). That could be the case of *F. affinis* and *A. neapolitana*, well established in the Mediterranean Sea (Kolb, 1998; Cervera et al., 2006) and only recently found in northern latitudes. This general migration pattern is inverted in the case of *A. gibba* and *O. bilamellata*, apparently expanding their distribution range southwards, which does not seem to be explained by the rise on the temperature of the water. Geographical expansion in these species can derive from a tolerance to a wider range of environmental conditions combined with the opportunity to migrate to empty niches previously occupied by species that have moved from their original habitats. Another possible explanation is to assume that, due to their great camouflage capability and the elusive living habits displayed, they may have been present in waters of Galicia before, but they have gone unnoticed.

The species presented have been found for the first time outside their theoretical distribution limits, a fact that has become customary in recent times (Philipart et al., 2011). Most of these species seem to be established in Galician waters, so an effort to continue monitoring faunal changes at a small scale will in the next few years enable us to confirm or refute the continuity of the populations, and their role in the ecosystems, enhancing general knowledge of the group and of the local ecosystems dynamics.

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