ONTGENETIC CHANGES IN THE SPICULE FORMATION AND THEIR POSSIBLE ROLE IN CHROMODORID OPISTHOBRANCHS (MOLLUSCA, CHROMODORIDIDAE)

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Abstract

Many dorid nudibranchs have calcareous spicules in their integument. These structures were lost during their evolution in some dorid lineages, as usually stated for most genera of the family Chromodorididae. Nevertheless, in this article, the presence of calcareous spicules is reported upon for the first time in 12 southern European species belonging to the genera Felimare and Felimida: Felimare villafranca, Felimare picta, Felimare orsinii, Felimare fontandraui, Felimare bilineata, Felimare cantabrica, Felimare tricolor, Felimida luteorosea, Felimida purpurea, Felimida krohni, Felimida luteopunctata and Felimida britoi. The spicules are arranged in the notum, lateral region of the body, rhinophores, gills and feet, and their morphology is very variable, even in the same species. Moreover, changes in size, shape and arrangement between juvenile and adult stages can be observed. Potential biological roles of these structures are discussed.

Key words: Felimare, Felimida, spicules, function, southern Europe

Introduction

Many families of dorid nudibranchs are characterized by the presence of spicules in the body wall, while others do not have them or they have been observed only in some individuals of these families. The family Chromodorididae Bergh, 1891 belongs to the latter group in which, to date, these structures have only been recorded in the genera Cadlina Bergh, 1878 and Cadlinella Thiele, 1931 (Rudman 1984) (although their inclusion in Chromodorididae is questionable, see below), and in some species belonging to other genera: thus, Gantès (1962) referred to the presence of spicules in the notum of juvenile specimens of Felimare villafranca (Risso, 1818) (referred to as Glossodoris gracilis); Thompson (1972) and Rudman (1999) observed these structures in post-larval specimens with direct development of Hyspelodoris bennetti (Angas, 1864) and Felimare zebra (Heilprin, 1884) (referred to as Hyspelodoris zebra), respectively; Schrödl & Millen (2001) observed spicules in adults of Tyrinna delicata (Abraham, 1877) (referred to as Tyrinna nobilis Bergh, 1898), and Rudman (1984, 1999) in juveniles of Noumea halicola (Burn, 1957) and Felimare zebra (the latter referred to as Hyspelodoris zebra).

Despite spicules being present in all these genera and species, either along the entire life cycle or only during the juvenile stage, Rudman (1998), according to previous notes by Schmekel & Portmann (1982), pointed out that the absence of spicules is one of the characters to consider in the diagnosis of the family Chromodorididae. This affirmation could be a consequence of the lack of studies about the presence of spicules in the genera of this family and also to the fact that phylogenetic relationships of Chromodorididae, mainly at its basal level, were not resolved. Thus, some authors consider Cadlina and Cadlinella within Chromodorididae (Thiele 1931; Boss 1982; Rudman 1984; Gosliner & Johnson 1999; Wilson & Lee 2005), while others consider them to belong to Cadliniidae (Bergh 1891; Bertsch 1977; Thompson & Brown 1984; Vaught 1989), or...
include Cadlina within Cadlinidae and Cadlinella as a sister to the remaining chromodorid species (Johnson & Gosliner 2012). Schrödl & Millen (2001) highlighted the problem, and molecular phylogenies support the hypothesis that Cadlina is not a member of Chromodorididae (Thollesson 1999; Grande et al. 2004; Turner & Wilson 2008). This view has been confirmed recently by Johnson (2011) after a comprehensive taxon sampling and outgroup selection.

Concerning the origin of the spicules, Ros (1976) suggested that they could be the remains of the shell or structures that have arisen secondarily as a response to the lack of hard structures. Nevertheless, Cattaneo-Vietti et al. (1993) considered the former hypothesis incorrect, because these structures are found not only in the notum but also in the foot, rhinophores and gills.

Regarding the function of spicules in ‘dorids’, a defensive role against predators has been proposed (Thompson 1960; Ros 1976, 1977; Thompson & Brown 1976; Garcia et al. 1986; Cattaneo-Vietti et al. 1993; Wägele & Willan 2000). According to Todd (1981), it is likely that spicules decrease the energetic value of these nudibranchs, making them less attractive to predators. Rudman (1984) suggested that the presence of spicules in the notum could make the nudibranch resemble the sponge upon which it preys, providing a camouflage against predators. Another proposed role is structural (Ros 1976), maintaining the stiffness and shape of the body (Vayssière 1901; Cattaneo-Vietti et al. 1993, 1995) and supporting the role of cariophyllidia tubercles (Foale & Willan 1987).

Penney (2006) carried out a morphological and functional study of the spicules of Cadlina luteomarginata McFarland, 1966, conducting experiments on their effectiveness as defensive structures by exposing specimens to attack by crabs and sea anemones. He concluded that spicules per se do not deter predators and proposed that their main role should be related to the support of the body.

Due to the lack of data about the presence/absence of spicules in the genera of the family Chromodorididae, and considering the utility that some authors give to these structures from a phylogenetic point of view (Valdés 2002), we made a study of some southern European species of the genera Felimare and Felimida according to the updated taxonomy of Johnson & Gosliner (2012), from which we have recorded the presence of spicules in all studied species. Moreover, we have studied the ontogenetic shifts that occur regarding their size, form and position, that may have different roles.

Material and methods
Most of the specimens were collected in western Mediterranean localities from the Iberian Peninsula: Punta del Vapor, Almuñécar (Granada) (03°43′41″W, 36°43′27″N) and Piedra del Hombre, La Herradura (Granada) (03°44′15″W, 36°43′29″N). The specimens of Felimare orsinii (Véran, 1846) were collected from the Columbretes Islands (0°40′E, 39°55′N) and Chafarinas Islands (Alboran Sea) (2°25′44″W, 35°10′46″N), both in W Mediterranean, while those of Felimida luteopunctata (Gantès, 1962) were collected in El Portil (Huelva, W Anlalusia, E Atlantic) (7°03′14.58″W, 37°12′30.7″N).

Genus Felimare Marcus & Marcus, 1867
Felimare villafraanca: 1 spec. of 610 μm; 1 spec. of 750 μm; 1 spec. of 2.5 mm; 1 spec. of 6 mm; 1 spec. of 11 mm; 1 spec. of 19 mm; 1 spec. of 23 mm; 1 spec. of 28 mm.
Felimare picta (Schultz, 1836): 1 spec. of 5 mm; 1 spec. of 13 mm; 1 spec. of 55 mm.
Felimare orsinii: 1 spec. of 8 mm; 1 spec. of 10 mm; 1 spec. of 18 mm.
Felimare fontandraui (Pruvot-Fol, 1951): 1 spec. of 8 mm; 1 spec. of 11 mm; 1 spec. of 22 mm.
Felimare bilineata (Pruvot-Fol, 1953): 1 spec. of 7 mm; 1 spec. of 21 mm; 1 spec. of 35 mm.
Felimare cantabrica (Bouchet & Ortea, 1980): 1 spec. of 7 mm; 1 spec. of 21 mm; spec. of 23 mm; 1 spec. of 45 mm.
Felimare tricolor (Cantraine, 1835): 1 spec. of 6 mm; 1 spec. of 15 mm; 1 spec. of 20 mm.

Genus Felimida Marcus, 1971
Felimida luteorosea (Rapp, 1827): 1 spec. of 18 mm; 1 spec. of 22 mm.
Felimida purpurea (Risso in Guérin, 1831): 1 spec. of 1 mm; 1 spec. of 5 mm; 1 spec. of 13 mm; 1 spec. of 20 mm.
Felimida krohni (Véran, 1846): 1 spec. of 5 mm; 1 spec. of 11 mm; 1 spec. of 22 mm.
Felimida luteopunctata: 1 spec. of 6 mm (preserved); 1 spec. of 13 mm (preserved).
Felimida britoi (Ortea & Pérez, 1983): 1 spec. of 5 mm; 1 spec. of 20 mm.

The specimens were photographed and measured in vivo (except those of Felimida luteopunctata, which were not collected directly by the authors); later they were frozen in seawater, being defrosted by the addition of ethanol (98%). Once defrosted, the rhinophores and gills were removed, and the remaining body was dissected laterally and longitudinally to remove the internal organs. The body wall was
divided into three parts: notum, lateral part of the body and the foot. Each part was mounted between a slide and a coverslide; a saturated solution of KOH was added for 5 min. Later they were photographed with a Sony P93 digital camera mounted on a light microscope, and the spicules were drawn with a camera lucida.

The terms used to define the relative abundance of the spicules, except in the interphase notum–hyponotum in which the amount is indicated, are as follows: very numerous, more than 100 per mm²; numerous, between 50 and 100 per mm²; scarce, between 10 and 50 per mm²; very scarce, fewer than 10 per mm².

The normality of the variables ‘number of spicules’ and ‘length of the specimen’ were tested with the Shapiro Wilk’s test for Felimare and Felimida specimens. In both cases data were normally distributed for the length of the animal and non-normally distributed for the number of spicules ($W = 0.84335, p < 0.05$ in Felimare and $W = 0.66055, p < 0.05$ in Felimida). Therefore, the Spearman’s non-parametric correlation was used in all cases.

Results

Spicules in the rhinophores, gills, notum, lateral region of the body and foot were observed in all the

Figure 1. Type of spicules in the studied species of Felimare and Felimida. Scale bars: 50 μm.
species. These structures, independently of their abundance, were distributed in a scattered manner, not constituting a continuous rigid structure. The number of the spicules varied depending on the length of the specimens and the region of the body.

Spicules were grouped into 12 categories according to their shape:

- Type A: thick spicules, with both tips pointed or slightly blunt, and usually slightly curved in their central part (Figure 1A).
- Type B: thin spicules, straight and with both tips pointed (Figure 1B).
- Type C: thick spicules with rounded tips and with tubercles on one of them (Figure 1C).

Figure 2. Changes in shape and disposition of the spicules of Felimare villafranca. Specimens of 750 μm (A), 6 mm (B), 19 mm (C) and 28 mm (D).

Figure 3. Changes in shape and disposition of the spicules of Felimida purpurea. Specimens of 1 mm (A), 5 mm (B) and 20 mm (C).
Type D: straight thick spicules with both tips rounded (Figure 1D).
Type E: curved spicules with both tips rounded (Figure 1E).
Type F: straight very thick spicules with both tips rounded and enlarged (Figure 1F).
Type G: straight very thick spicules with many tubercles and with rounded tips (Figure 1G).
Type H: slightly curved and very thick spicules with rounded tips (Figure 1H).
Type I: straight thin spicules with many tubercles (Figure 1I).
Type J: more or less spherical spicules with many thick tubercles (Figure 1J).
Type K: spherical spicules with small tubercles (Figure 1K).
Type L does not occur in Tables I–XII.

A shift could be observed in the number of spicules of the notum, but also in the shape and arrangement, from juveniles to adults (Figures 2 and 3). Thus, two small examined specimens of Felimare villafranca (750 μm) and Felimida purpurea (1 mm) had a total amount of 72 and 52 spicules of Type A, respectively. These were arranged mainly on the edge of the notum, with one of the tips usually oriented towards the outside (Figure 3A–C). Neither of the two specimens had developed mantle dermal formations (MDFs). In slightly larger specimens of F. villafranca (2.5 mm) and F. purpurea (5 mm), the number of spicules did not vary significantly (80 and 50, respectively), but an increase in the size could be detected (Figure 4) and the MDFs could be observed. Moreover, these larger spicules were placed in the interphase notum–hyponotum. In the remaining specimens, the number of spicules in the interphase decreased as the size of the animal increased. Thus, in specimens of F. villafranca longer than 20 mm the number of spicules in this interphase was lower than 10 (Figure 5) and they were not observed in specimens of F. purpurea longer than 13 mm. This decrease occurred in all studied Felimare species in such a way that there was a statistically significant negative correlation ($r_s = -0.90, p < 0.05; n = 19$) between the size of the animal and the number of spicules in the interphase notum–hyponotum (Figure 6). The same situation was observed in specimens of Felimida (Figure 7), with the correlation also being
negative and statistically significant ($r_1 = -0.838$, $p < 0.05$; $n = 9$; Figure 7).

In adult specimens of *Felimare villafranca*, *Felimare tricolor*, *Felimare bilineata* and *Felimare cantabrica* the spicules of the interphase notum–hyponotum were substituted with smaller spicules of Type F (Figure 8A) distributed throughout the entire notum and lateral region of the body; the spicules of the rhinophores were thicker at the base and with a more or less uniform transversal section and rounded tips (Figure 8B), and thinner and fusiform at the apex (Figure 8C). Finally, the spicules of the inner region of the body wall of specimens larger than 1 mm of the species of *Felimida* were generally spherical-shaped with many tubercles (Figure 8D,E).

In all studied species, except in *Felimida luteorosea*, small spicules of Type L could be observed sparsely distributed in the body wall, rhinophores and gills. Their height ranged between 5 and 15 μm (Figure 1L).

Detailed information about spicule shape, distribution, size (minimum and maximum, mean and standard deviation, and ‘$n$’ = the number of spicules), and abundance in the studied species are provided in Tables I–XII (see also Supplementary material).

**Discussion**

Calcereous spicules are present in some family-taxa of dorid nudibranchs, but are absent in others, and according to Schmekel & Portmann (1982), Rudman (1984, 1998) and Valdés (2002) the genera *Hypselodoris* Stimpson, 1855 and *Chromodoris* Alder & Hancock, 1855 (many of the species previously considered to be in those genera, including all the species studied by us, have recently been transferred to the genera *Felimare* and *Felimida*) lack these structures. However, our results reject this view. It is likely that the idea of a lack of spicules results from only superficial studies of the external adult morphology of these groups, as spicules have been observed in the rhinophores, gills, notum, lateral part of the body and foot of the examined specimens in both genera.

The shape, arrangement and high number of spicules from the edge of the notum and the interphase notum–hyponotum in the early juvenile stages,
### Table I. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, \(n\) = number of spicules) and abundance in six specimens of *Felimare villafranca*.

| Size of the specimen | Rhinophores | Gills | Anterior edge of the notum | Notum | Interphase notum–hyponotum | Hyponotum | Foot |
|----------------------|-------------|-------|---------------------------|-------|---------------------------|-----------|------|
| 750 \(\mu\)m       | Not developed | Not developed | Type A; 72 spicules; between 75 and 160 \(\mu\)m, mean 111 ± 26.1 \(\mu\)m (\(n=10\)) | Not observed | Not observed | Type B; very scarce; between 70 and 95 \(\mu\)m, mean 80 ± 10.8 \(\mu\)m (\(n=4\)) |
| 2.5 mm               | Not studied | Type B; scarce; between 65 and 110 \(\mu\)m, mean 81.25 ± 23.2 \(\mu\)m (\(n=3\)) | Type A; 80 spicules; between 140 and 300 \(\mu\)m, mean 219 ± 45.2 \(\mu\)m (\(n=10\)) | Not observed | Not observed | Type A; scarce; between 130 and 230 \(\mu\)m, mean 185.4 ± 39.2 \(\mu\)m (\(n=10\)) |
| 6 mm                 | Type B; very numerous; between 65 and 140 \(\mu\)m, mean 104.3 ± 27.1 \(\mu\)m (\(n=7\)) | Type B; scarce; between 80 and 190 \(\mu\)m, mean 124 ± 49.3 \(\mu\)m (\(n=5\)) | Types B and D; very scarce; between 100 and 170 \(\mu\)m, mean 133.3 ± 28 \(\mu\)m (\(n=3\)) | Types B and D; very scarce; central region; between 70 and 180 \(\mu\)m, mean 116.6 ± 46.2 \(\mu\)m (\(n=10\)) | Types A and C; 72 spicules; between 190 and 340 \(\mu\)m, mean 266 ± 52.7 \(\mu\)m (\(n=10\)) | Types B and D; very scarce; between 40 and 130 \(\mu\)m, mean 84.7 ± 30.6 \(\mu\)m (\(n=8\)) | Types A and D; scarce; more concentrated in the tail; between 170 and 360 \(\mu\)m, mean 245.5 ± 66.5 \(\mu\)m (\(n=10\)) |
| 11 mm                | Type B; very numerous; between 60 and 195 \(\mu\)m, mean 128.1 ± 35.5 \(\mu\)m (\(n=10\)) | Types B and D; very numerous; arranged perpendicular to the edge; between 120 and 240 \(\mu\)m, mean 175.5 ± 39.2 \(\mu\)m (\(n=10\)) | Types B, C and D; very numerous; between 70 and 135 \(\mu\)m, mean 104.5 ± 20.2 \(\mu\)m (\(n=10\)) | Types B; very scarce; between 70 and 135 \(\mu\)m, mean 104.5 ± 20.2 \(\mu\)m (\(n=10\)) | Types A; 32 spicules; between 240 and 315 \(\mu\)m, mean 281.5 ± 24 \(\mu\)m (\(n=10\)) | Types B and D; scarce; between 100 and 140 \(\mu\)m, mean 120.5 ± 14.9 \(\mu\)m (\(n=10\)) | Types B and D; scarce; between 100 and 265 \(\mu\)m, mean 170.5 ± 64.1 \(\mu\)m (\(n=10\)) |
| 19 mm                | Types B and D; very numerous; between 100 and 235 \(\mu\)m, mean 132.5 ± 41.9 \(\mu\)m (\(n=10\)) | Types D and E; scarce; between 95 and 240 \(\mu\)m, mean 154 ± 42 \(\mu\)m (\(n=10\)) | Types B and D; very numerous; arranged perpendicular to the edge, the smaller ones in the outer region; between 50 and 265 \(\mu\)m, mean 176.5 ± 63.9 \(\mu\)m (\(n=10\)) | Types B and D; very numerous; arranged perpendicular to the edge, the smaller ones in the outer region; between 50 and 265 \(\mu\)m, mean 176.5 ± 63.9 \(\mu\)m (\(n=10\)) | Types A and C; 7 spicules; between 195 and 370 \(\mu\)m, mean 267.5 ± 75 \(\mu\)m (\(n=7\)) | Types F; very numerous; between 45 and 180 \(\mu\)m, mean 94.5 ± 33.5 \(\mu\)m (\(n=10\)) | Types B and D; scarce; between 65 and 220 \(\mu\)m, mean 182.5 ± 70.1 \(\mu\)m (\(n=10\)) |
| 28 mm                | Types B and D; very numerous; between 80 and 210 \(\mu\)m, mean 140 ± 36.8 \(\mu\)m (\(n=10\)) | Types D and E; scarce; between 70 and 260 \(\mu\)m, mean 155 ± 106 \(\mu\)m (\(n=10\)) | Types B and D; very numerous; arranged perpendicular to the edge, the smaller ones in the outer region; between 80 and 180 \(\mu\)m, mean 132.5 ± 35.7 \(\mu\)m (\(n=10\)) | Types F; scarce; between 85 and 150 \(\mu\)m, mean 102 ± 23.7 \(\mu\)m (\(n=10\)) | Types A; 2 spicules; 370 and 380 \(\mu\)m in length, respectively | Types F; very numerous; between 80 and 140 \(\mu\)m, mean 104 ± 22.1 \(\mu\)m (\(n=10\)) | Types B and D; scarce; between 60 and 315 \(\mu\)m, mean 118 ± 88.8 \(\mu\)m (\(n=10\)) |
Table II. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in three specimens of *Felimare picta*.

| Region of the body | Size of the specimen | Rhinophores | Gills | Anterior edge of the notum | Notum | Interphase notum–hyponotum | Hyponotum | Foot |
|--------------------|----------------------|-------------|-------|--------------------------|-------|---------------------------|-----------|------|
|                    |                      |             |       |                          | Not observed | Types A and C; 50 spicules; between 235 and 360 μm, mean 310.7 ± 53.5 μm (n = 7) | Type A; very scarce; between 220 and 280 μm, mean 248.1 ± 21.7 μm (n = 8) | Types D and C; scarce; between 140 and 310 μm, mean 234 ± 55.5 μm (n = 10) |
| 5 mm               | Not studied          | Not studied |       |                          | Not observed | Types D and F; very scarce; between 30 and 120 μm, mean 52.7 ± 29 μm (n = 9) | Not observed | Types D and F; very scarce; between 20 and 80 μm, mean 40.25 ± 7.9 μm (n = 4) | Types D and F; very scarce; between 20 and 75 μm, mean 27 ± 6.4 μm (n = 6) |
| 13 mm              | Type B; numerous; between 35 and 170 μm, mean 97 ± 34.8 μm (n = 10) | Type B; very scarce; between 40 and 115 μm, mean 72 ± 29 μm (n = 6) | Not observed | Types D and F; very scarce; between 30 and 120 μm, mean 52.7 ± 29 μm (n = 9) | Not observed | Types D and F; very scarce; between 20 and 80 μm, mean 40.25 ± 7.9 μm (n = 4) | Types D and F; very scarce; between 20 and 75 μm, mean 27 ± 6.4 μm (n = 6) |
| 55 mm              | Types B and E; very numerous; between 25 and 210 μm, mean 113 ± 57.3 μm (n = 10) | Types B and E; scarce; between 80 and 95 μm, mean 86.6 ± 7.6 μm (n = 3) | Not observed | Types B; very numerous; between 15 and 80 μm, mean 46.3 ± 17.32 μm (n = 10) | Types B; very numerous; between 15 and 80 μm, mean 46.3 ± 17.32 μm (n = 10) | Types F; very scarce; between 20 and 75 μm, mean 27 ± 6.4 μm (n = 6) | Types B; scarce; between 30 and 38 μm, mean 34 ± 5.6 μm (n = 2) | Types F; numerous; between 25 and 150 μm, mean 64.7 ± 42.9 μm (n = 7) |


Table III. Spicules shape distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and the abundance in three specimens of *Felimare ornitii*.

| Size of the specimen | Rhinophores | Gills | Notum | Interphase notum–hyponotum | Hyponotum | Foot |
|----------------------|-------------|-------|-------|-----------------------------|-----------|------|
| 8 mm                 | Type B; very scarce; between 40 and 110 μm, mean 70 ± 23 μm (n = 7) | Types B and E; very scarce between 80 and 160 μm, mean 111 ± 25.5 μm (n = 10) | Types B and D; very scarce; between 90 and 200 μm, mean 140 ± 27.6 μm (n = 10) | Type A; 95 spicules; between 190 and 400 μm, mean 315 ± 69.48 μm (n = 10) | Type D; numerous; between 120 and 210 μm, mean 154 ± 24.1 μm (n = 10) | Types A and D; very numerous in the posterior part, scarce in the central part and very scarce in the anterior part; between 80 and 300 μm, mean 201 ± 77.2 μm (n = 10) |
| 10 mm                | Types B and E; scarce; between 100 and 195 μm, mean 133 ± 32 μm (n = 10) | Types B and E; very scarce between 70 and 190 μm, mean 114.5 ± 40.3 μm (n = 5) | Types B and D; very scarce; between 70 and 150 μm, mean 97 ± 30.8 μm (n = 10) | Type A; 80 spicules; between 210 and 420 μm, mean 331 ± 53.6 μm (n = 10) | Type D; numerous; between 60 and 120 μm, mean 99 ± 26.47 μm (n = 10) | Types A and D; very numerous in the posterior part, scarce in the central part and very scarce in the anterior part; between 55 and 160 μm, mean 114 ± 37.9 μm (n = 10) |
| 18 mm                | Type B; very scarce; between 120 and 160 μm, mean 140 ± 16.3 μm (n = 10) | Types B and E; very scarce between 100 and 200 μm, mean 139 ± 34.1 μm (n = 10) | Types B and D; very scarce; between 90 and 160 μm, mean 121 ± 18.5 μm (n = 10) | Type A; 110 spicules; between 180 and 420 μm, mean 290 ± 84 μm (n = 10) | Type D; numerous; between 60 and 150 μm, mean 120 ± 25 μm (n = 10) | Types A and D; very numerous in the posterior part, scarce in the central part and very scarce in the anterior part; between 130 and 330 μm, mean 253 ± 62.1 μm (n = 10) |

which lack chemical defences or are not yet fully developed, lead us to propose the hypothesis of a possible deterrent role which would be in agreement with the defensive role suggested for these structures by Thompson (1960), Ros (1976, 1977), Thompson & Brown (1976), García et al. (1986), Cattaneo-Vietti et al. (1993) and Wägele & Willan (2000). The decrease in the number of spicules in the interphase

Table IV. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in three specimens of *Felimare fontandrai*.

| Size of the specimen | Rhinophores | Gills | Notum | Interphase notum–hyponotum | Hyponotum | Foot |
|----------------------|-------------|-------|-------|-----------------------------|-----------|------|
| 8 mm                 | Type B; numerous; between 80 and 220 μm, mean 146 ± 43.89 μm (n = 10) | Type B; scarce; spindle-like; between 120 and 280 μm, mean 177 ± 54.98 μm (n = 10) | Not observed | Types D and E; very scarce; between 25 and 55 μm, mean 38.7 ± 9.8 μm (n = 10) | Type A; 41 spicules; between 220 and 600 μm, mean 438 ± 125.8 μm (n = 10) | Types B and D; very scarce; between 25 and 55 μm, mean 38.6 ± 11.7 μm (n = 10) |
| 11 mm                | Type B; numerous; between 75 and 170 μm, mean 126.5 ± 29.5 μm (n = 10) | Type B; scarce; between 85 and 220 μm, mean 146 ± 50.9 μm (n = 10) | Not observed | Type D; very scarce; between 25 and 135 μm, mean 62 ± 31.5 μm (n = 10) | Type A; 16 spicules; between 220 and 565 μm, mean 394.5 ± 126.8 μm (n = 10) | Types B and D; very scarce; between 35 and 350 μm, mean 138 ± 103.4 μm (n = 10) |
| 22 mm                | Type B; very numerous; between 45 and 180 μm, mean 118 ± 40.7 μm (n = 10) | Types B and D; scarce; between 70 and 160 μm, mean 98.5 ± 32.3 μm (n = 10) | Not observed | Type D; very scarce; between 40 and 210 μm, mean 100 ± 43.5 μm (n = 10) | Not observed | Types B and D; scarce; concentrated at the tail mainly; between 50 and 180 μm, mean 109 ± 34.3 μm (n = 10) |
Table V. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in two specimens of *Felimare bilineata*.

| Size of the specimen | Rhinophores | Gills | Anterior edge of the notum | Notum | Interphase notum-hyponotum | Hyponotum | Foot |
|----------------------|-------------|-------|---------------------------|-------|---------------------------|-----------|------|
| 7 mm                 | Types B and D; very numerous; between 50 and 80 μm, mean 70.7 ± 12 μm (n = 7) | Types B and D; very scarce; between 80 and 95 μm, mean 86.6 ± 7.63 μm | Not observed | Not observed | Type A; 28 spicules; between 170 and 385 μm, mean 279.1 ± 83.1 μm (n = 10) | Types D and F; scarce; between 40 and 85 μm, mean 69 ± 13.9 μm (n = 10) | Types B; scarce; between 80 and 290 μm, mean 182.5 ± 60.5 μm (n = 10) |
| 21 mm                | Type D; very numerous; between 85 and 130 μm, mean 94 ± 13.7 μm (n = 10) | Types D; very scarce; between 50 and 95 μm, mean 68.5 ± 2.4 μm (n = 10) | Not observed | Type F; very numerous; between 70 and 100 μm, mean 57 ± 8.2 μm (n = 10) | Not observed | Type F; very numerous; between 55 and 70 μm, mean 62 ± 4.4 μm (n = 10) | Type F; very numerous; between 40 and 75 μm, mean 53 ± 10.8 μm (n = 10) |

Table VI. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in four specimens of *Felimare cantabrica*. MDF = mantle dermal formations.

| Size of the specimen | Rhinophores | Gills | Anterior edge of the notum | Notum | Interphase notum-hyponotum | Hyponotum | Foot |
|----------------------|-------------|-------|---------------------------|-------|---------------------------|-----------|------|
| 7 mm                 | Type B; very numerous; between 55 and 180 μm, mean 108.5 ± 39.7 μm (n = 10) | Type B; numerous; between 50 and 160 μm, mean 85 ± 39.3 μm (n = 6) | Not observed | Type B; scarce; between 25 and 85 μm, mean 60.5 ± 19.7 μm (n = 10) | Type C; very scarce; posterior region; arranged among the MDFs and perpendicular to the outer edge; between 370 and 400 μm, mean 385 ± 14.1 μm (n = 5) | Types A and C; 42 spicules; between 230 and 380 μm, mean 280 ± 39.7 μm (n = 10) | Types D; scarce; between 45 and 130 μm, mean 68 ± 25 μm (n = 10) | Types B, C and D; very scarce; between 50 and 245 μm, mean 144 ± 70.9 μm (n = 10) |
| 21 mm                | Type B; very numerous; between 85 and 135 μm, mean 115 ± 25.1 μm (n = 10) | Type B; numerous; between 30 and 130 μm, mean 81.5 ± 30.7 μm (n = 10) | Not observed | Type D; very numerous; between 25 and 165 μm, mean 78.5 ± 52.1 μm (n = 10) | Types A and B; 2 spicules; between 35 and 85 μm, mean 52.2 ± 14.9 μm (n = 10) | Type D and F; very numerous; between 35 and 85 μm, mean 52.4 ± 14.9 μm (n = 10) | Types C, D and F; very numerous; between 40 and 180 μm, mean 76.5 ± 47.9 μm (n = 10) | Types B, C and D; very scarce; between 50 and 245 μm, mean 144 ± 70.9 μm (n = 10) |
| 23 mm                | Types B and D; very numerous; between 80 and 270 μm, mean 152.5 ± 55.7 μm (n = 10) | Types B and D; numerous; between 35 and 150 μm, mean 84.5 ± 11.7 μm (n = 10) | Not observed | Types D and F; very numerous; between 50 and 170 μm, mean 84.5 ± 35.7 μm (n = 10) | Not observed | Types D and F; very numerous; between 40 and 120 μm, mean 68 ± 26.1 μm (n = 10) | Types D and F; very numerous; between 50 and 205 μm, mean 112.5 ± 173.1 μm (n = 10) | Types D and F; very numerous; between 50 and 205 μm, mean 112.5 ± 173.1 μm (n = 10) |
| 45 mm                | Types B and D; very numerous; between 40 and 130 μm, mean 85.5 ± 26.6 μm (n = 10) | Types D; very scarce; between 40 and 75 μm, mean 61 ± 11.7 μm (n = 10) | Not observed | Type F; very numerous; between 40 and 75 μm, mean 52.5 ± 14.5 μm (n = 10) | Not observed | Type F; very numerous; between 35 and 70 μm, mean 51 ± 14.5 μm (n = 10) | Types D and F; very numerous; between 30 and 55 μm, mean 45.5 ± 8.3 μm (n = 10) | Types D and F; very numerous; between 30 and 55 μm, mean 45.5 ± 8.3 μm (n = 10) |
The efficiency of chemical defences increases, would support this hypothesis.

One of the regions of the body which is more exposed to predators is the notum, particularly in species in which the edge of the notum is flap-like or has a straight, prominent edge. Thus, most of the studied species of *Felimare* have many small spicules in both regions. Based on species of sponges and anthozoans, Koehl (1982) conducted a study on the mechanical design of spicules and their role in strengthening connective tissue in animals.

| Size of the specimen | Rhinophores | Gills | Anterior edge of the notum | Notum | Interphase notum–hyponotum | Hyponotum | Foot | Region of the body |
|----------------------|-------------|-------|----------------------------|-------|----------------------------|-----------|------|--------------------|
| **6 mm**             | Type B; very numerous; between 60 and 200 μm, mean 155 ± 38.9 μm (n = 5) | Types B and E; very numerous; between 70 and 126 ± 42.5 μm (n = 10) | Not observed | Type F; very scarce; between 60 and 85 μm, mean 74 μm (n = 10) | Types A and C; very numerous; 64 spicules; between 220 and 350 μm, mean 287.5 ± 42.3 μm (n = 10) | Types F; scarce; between 60 and 80 μm, mean 70 ± 5.7 μm (n = 10) | Types A and D; very scarce; between 160 and 545 μm, mean 304.4 ± 135.4 μm (n = 10) | **Spicule formation in chromodorid opisthobranchs** |
| **15 mm**             | Type D; very numerous; between 65 and 150 μm, mean 95.5 ± 14.2 μm (n = 10) | Types B and D; very numerous; between 60 and 98.5 ± 19.8 μm (n = 10) | Not observed | Type F; very numerous; between 55 and 100 μm, mean 68 ± 13.5 μm (n = 10) | Type A; 8 spicules; between 310 and 410 μm, mean 360.6 ± 35.2 μm (n = 8) | Type F; very numerous; between 40 and 60 μm, mean 47.5 ± 6.3 μm (n = 10) | Type F; very numerous; between 35 and 140 μm, mean 74.5 ± 43.8 μm (n = 10) | **Table VII. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in three specimens of *Felimare tricolor*.** |
| **20 mm**             | Type B and D; very numerous; between 60 and 125 μm, mean 98.5 ± 19.8 μm (n = 10) | Types B and D; very numerous; between 50 and 110 μm, mean 73.5 ± 17.6 μm (n = 10) | Not observed | Type F; very numerous; between 45 and 100 μm, mean 63.5 ± 17.32 μm (n = 10) | Type F; very numerous; between 35 and 75 μm, mean 50.5 ± 13.6 μm (n = 10) | Type F; very numerous; between 35 and 65 μm, mean 45.5 ± 10.1 μm (n = 10) | **Table VIII. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in two specimens of *Felimida luteorosea*.** |

| Size of the specimen | Rhinophores | Gills | Notum | Hyponotum | Foot | Inner side of the body wall |
|----------------------|-------------|-------|-------|-----------|------|----------------------------|
| **18 mm**             | Types B and D; very numerous; between 70 and 135 μm, mean 108.5 ± 27.5 μm (n = 10) | Type B; scarce; between 70 and 100 μm, mean 88 ± 10.3 μm (n = 10) | Type D; very numerous; mainly in anterior region; between 40 and 85 μm, mean 67 ± 12.5 μm (n = 10) | Type D; very numerous; between 60 and 85 μm, mean 71 ± 11 μm (n = 10) | Type B; very numerous; between 65 and 100 μm, mean 85.5 ± 11 μm (n = 10) | Types I and J; very numerous; between 15 to 25 μm, mean 19 ± 3.1 μm (n = 10) (Figure 8E) |
| **22 mm**             | Types B and D; very numerous; between 50 and 150 μm, mean 95.4 ± 26.9 μm (n = 10) | Type G; scarce; between 50 and 100 μm, mean 80.5 ± 14 μm (n = 10) | Type D; very numerous; mainly in anterior region of the edge; between 70 and 110 μm, mean 86.5 ± 12.7 μm (n = 10) | Not observed | Type B; very numerous; between 65 and 95 μm, mean 78.5 ± 11.3 μm (n = 10) | Types I and J; very numerous; between 10 to 15 μm, mean 13.5 ± 2.4 μm (n = 10) |

*Table VIII. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in two specimens of *Felimida luteorosea*.**
### Table IX. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, 'n' = number of spicules) and abundance in four specimens of Felimida purpurea.

| Region of the body | Size of the specimen | Rhinophores | Gills | Edge of the notum | Notum | Foot | Inner side of the body wall |
|--------------------|----------------------|-------------|-------|--------------------|-------|------|-----------------------------|
|                    | 1 mm                 | Not developed | Not developed | Type A; 52 spicules; between 100 and 180 µm, mean 156 ± 28.7 µm (n = 10) | Type B; very scarce; between 55 and 60 µm, mean 58.3 ± 2.8 µm (n = 3) | Not observed | |
|                    | 5 mm                 | Type B; very numerous; between 65 and 110 µm, mean 83.5 ± 13.9 µm (n = 10) | Type B; very scarce; only 1 spicule of 85 µm | Anterior region, Type B; numerous; mainly arranged perpendicularly to the edge; between 80 and 125 µm, mean 99.5 ± 18.9 µm (n = 10) | Type B and D; scarce; between 55 and 340 µm, mean 203.5 ± 114.8 µm (n = 10) | Types I and J; very numerous; between 10 and 30 µm, mean 21.5 ± 10.6 µm (n = 10) | |
|                    | 13 mm                | Type B; very numerous; between 70 and 125 µm, mean 97 ± 22 µm (n = 10) | Not studied | Anterior region; Types B, D and E; very numerous and mainly arranged perpendicularly to it; between 75 and 170 µm; mean 118 ± 32.5 µm (n = 10). Towards the inner region (two morphotypes): Type B; numerous; between 150 and 360 µm, mean 290 ± 72.2 µm (n = 10). Type G; scarce; between 350 and 550 µm, mean 459 ± 51.9 µm (n = 10) | Type B and D; very scarce; between 45 and 220 µm; mean 103 ± 55.4 µm (n = 10). Type G; very scarce; between 240 and 370 µm, mean 306 ± 66.1 µm (n = 9) | Not observed | Types B and D; very numerous; between 20 and 25 µm, mean 23 ± 2.5 µm (n = 10) |
|                    | 20 mm                | Type B; very numerous; between 85 and 145 µm, mean 116 ± 18 µm (n = 10) | Type B; very scarce; between 105 and 280 µm, mean 168 ± 79.1 µm (n = 3) | Anterior region, from the outer region towards the inner region (three morphotypes). Types B and D; very numerous; arranged perpendicularly to the edge; between 100 and 150 µm, mean 110 ± 15.2 µm (n = 10). Types B and H; numerous; between 90 and 380 µm, mean 222.5 ± 105.6 µm (n = 10). Type G; very scarce; between 225 and 480 µm, mean 376.8 ± 99.8 µm (n = 8) | Type B and D; scarce; between 80 and 230 µm; mean 152 ± 40.7 µm (n = 10) | Type J; very numerous; between 30 and 35 µm, mean 30.5 ± 2.8 µm (n = 10) (Figure 8D) | }

(Figure 8D)
Table X. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in three specimens of Felimida krohni.

| Size of the specimen | Region of the body                      | Rhinophores                      | Gills                        | Edge of the notum               | Notum                      | Hyponotum                   | Foot                        | Inner side of the body wall |
|----------------------|----------------------------------------|----------------------------------|------------------------------|---------------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| 5 mm                 | Anterior; Type B; very scarce; between 65 and 85 μm, mean 75 ± 6.3 μm (n = 6) | Not studied                      | Type A; 23 spicules; interphe notum–hyponotum; between 270 and 360 μm, mean 299 ± 28 μm (n = 10) | Types A and B; very scarce; mainly arranged in the tail; between 130 and 260 μm, mean 200 ± 47.1 μm (n = 10) | Types I and J; very numerous; between 12 and 22 μm, mean 17.4 ± 2.9 μm (n = 10) |
|                      | Remaining parts: Type C; very scarce; arranged perpendicularly to the edge; between 210 and 360 μm, mean 302 ± 49.6 μm (n = 10) | (n = 10)                          | (n = 10)                      | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     | (n = 10)                     |
|                      | 10A). Thus, these detach from the body and become extended away from the environment, are held outside (Figure 8C). | Types B and D; very numerous; between 60 and 100 μm, mean 74 ± 11 μm (n = 10) | Types B and D; very scarce; between 70 and 95 μm, mean 81.6 ± 12.5 μm (n = 3) | Types B and D; between 50 and 100 μm, mean 84 ± 16.2 μm (n = 10) | Types B and D; between 50 and 105 μm, mean 76 ± 14.6 μm (n = 10) | Types J; very numerous; between 15 and 20 μm, mean 17.8 ± 3.5 μm (n = 10) | Types I and J; very numerous; between 15 and 35 μm, mean 22.5 ± 10.6 μm (n = 10) |
|                      | (1981) and Penney (2006). The arrangement of the spicules in the rhinophores, more pointed in the apical region (Figure 8C) and higher in number compared with those of the gills (Figure 10B), may play a possible deterrent role against these attacks or, as the above authors point out, represent an indirect system to decrease the energetic value due to the great number of spicules and, consequently, be rejected by the fish as food. | (n = 10)                          | (n = 3)                       | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     |
|                      | Fish are one of the most important groups of predators of nudibranchs according to Todd (1981) and Penney (2006). The arrangement of the spicules in the rhinophores, more pointed in the apical region (Figure 8C) and higher in number compared with those of the gills (Figure 10B), may play a possible deterrent role against these attacks or, as the above authors point out, represent an indirect system to decrease the energetic value due to the great number of spicules and, consequently, be rejected by the fish as food. | (n = 10)                          | (n = 10)                      | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     |
|                      | A second role attributed to the spicules is structural (Ros 1976; Cattaneo-Vietti et al. 1993; Foale & Willan 1987), but their scattered arrangement in the studied species of the genera Felimare and Felimida lead us to propose that their importance in this role is less than in those dorids in which bundles are accumulated. In any case, their arrangement and the higher number in some regions of the body, as in the anterior part and the edge of the notum of the Felimida species, seem to significantly contribute to the stiffness of the animal as well. In this region, the spicules are mostly arranged forming a small angle with respect to the edge or perpendicular to it, and their size increases towards the inside (Figure 10C). | (n = 10)                          | (n = 10)                      | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     |
|                      | According to Penney (2006), the role of the spicules of the rhinophores and gills is to maintain stiffness. | Types B and D; very numerous; between 45 and 170 μm, mean 107 ± 36.6 μm (n = 10) | Types B and D; very scarce; between 70 and 100 μm, mean 85 ± 14.7 μm (n = 4) | Types B and D; between 50 and 125 μm, mean 83.5 ± 21 μm (n = 10) | Types B and D; between 65 and 125 μm, mean 91 ± 22.4 μm (n = 10) | Types A, B, D and G; scarce; between 60 and 170 μm; mean 110 ± 37.2 μm (n = 10) | Types I and J; very numerous; between 15 and 35 μm, mean 22.5 ± 10.6 μm (n = 10) |
|                      | (1981) and Penney (2006). The arrangement of the spicules in the rhinophores, more pointed in the apical region (Figure 8C) and higher in number compared with those of the gills (Figure 10B), may play a possible deterrent role against these attacks or, as the above authors point out, represent an indirect system to decrease the energetic value due to the great number of spicules and, consequently, be rejected by the fish as food. | (n = 10)                          | (n = 10)                      | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     |
|                      | A second role attributed to the spicules is structural (Ros 1976; Cattaneo-Vietti et al. 1993; Foale & Willan 1987), but their scattered arrangement in the studied species of the genera Felimare and Felimida lead us to propose that their importance in this role is less than in those dorids in which bundles are accumulated. In any case, their arrangement and the higher number in some regions of the body, as in the anterior part and the edge of the notum of the Felimida species, seem to significantly contribute to the stiffness of the animal as well. In this region, the spicules are mostly arranged forming a small angle with respect to the edge or perpendicular to it, and their size increases towards the inside (Figure 10C). | (n = 10)                          | (n = 10)                      | (n = 10)                        | (n = 10)                    | (n = 10)                     | (n = 10)                     |
Table XI. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in two specimens of *Felimida luteopunctata*.

| Size of the specimen | Rhinophores | Gills | Edge of notum | Foot | Inner side of the body wall |
|----------------------|-------------|-------|---------------|------|-----------------------------|
| 6 mm (preserved)     | Type B; very scarce; between 80 and 140 μm, mean 107.1 ± 22 μm (n = 7) | Type B; numerously arranged perpendicularly to it; between 85 and 115 μm, mean 99 ± 9.3 μm (n = 10) | Types B, D and E; numerous; between 80 and 140 μm, mean 109.5 ± 18.9 μm (n = 10) | Type J; very numerous; between 10 and 15 μm, mean 13.4 ± 2.1 μm (n = 10) |
|                      | Type B; numerous; between 25 and 75 μm, mean 53 ± 14.3 μm (n = 10)       | Type B; very scarce; between 25 and 40 μm, mean 12.5 ± 5 μm (n = 10) | Types B, D and E; numerous; between 80 and 140 μm, mean 109.5 ± 18.9 μm (n = 10) | Type K; numerous; between 25 and 45 μm, mean 35.3 ± 5.85 μm (n = 10) |

Table XII. Spicule shape, distribution, size (minimum and maximum, mean and standard deviation, ‘n’ = number of spicules) and abundance in two specimens of *Felimida britoi*.

| Size of the specimen | Rhinophores | Gills | Edge of notum | Notum | Foot | Inner side of the body wall |
|----------------------|-------------|-------|---------------|-------|------|-----------------------------|
| 5 mm                 | Type B; very scarce; between 40 and 115 μm, mean 68.5 ± 20.5 μm (n = 10) | Type B; very scarce; (two spicules); basal region: Type I; numerous; between 10 and 60 μm, mean 30.4 ± 16.3 μm (n = 10) | Anterior region: Type B; numerous and mainly arranged perpendicularly to it; between 35 and 105 μm, mean 72.5 ± 17.5 μm (n = 10). Towards the inner region: Types A and C; eight spicules between 200 and 280 μm, mean 243.7 ± 27.7 μm (n = 8). Posterior region: Type C; eight spicules between 270 and 590 μm, mean 408.7 ± 107.7 μm (n = 8) | Type A; three spicules; between 440 and 520 μm, mean 486 ± 41.6 μm (n = 3) | Types B and D; very numerous; between 170 and 270 μm, mean 122 ± 29.2 μm (n = 10) | Only anterior region: Type I; very numerous; between 25 and 45 μm, mean 28.3 ± 6.23 μm (n = 10). Rest: Type J; very numerous; between 15 and 20 μm, mean 18 ± 4.8 μm (n = 10). Type K; scarce to numerous depending on the zone; between 20 and 30 μm, mean 25.6 ± 3.3 μm (n = 10). |
|                      | Type B; very scarce; (two spicules); basal region: Type I; numerous; between 25 and 70 μm, mean 49.5 ± 14.2 μm (n = 10) | Type B; Anterior region: numerous and mainly arranged perpendicularly to it; between 70 and 100 μm, mean 85 ± 11.5 μm (n = 10). Posterior region: very scarce; four spicules between 50 and 85 μm, mean 75 ± 16.8 μm (n = 4) | Types B and D; numerous; between 80 and 130 μm, mean 94.5 ± 16 μm (n = 10) | Types B and D; very numerous; between 80 and 130 μm, mean 94.5 ± 16 μm (n = 10) | Types B and D; very numerous; between 80 and 130 μm, mean 94.5 ± 16 μm (n = 10) | Only anterior region: Type I; very numerous; between 25 and 45 μm, mean 31 ± 7.7 μm (n = 10). Rest: Type J; very numerous; between 12 and 25 μm, mean 17.2 ± 3.8 μm (n = 10). Type K; scarce to numerous depending on the zone; between 25 and 30 μm, mean 25.3 ± 2.5 μm (n = 10). |
| 22 mm                | Type B; scarce; between 35 and 90 μm, mean 75.5 ± 16.4 μm (n = 10) | Type I; very scarce; (one spicule); basal region: 45 μm | Anterior region: Type B; scarce and mainly arranged perpendicularly to it; between 60 and 100 μm, mean 83 ± 12.5 μm (n = 10). Towards the inner region: Types A, C and D; very scarce; between 180 and 360 μm, mean 237 ± 64.9 μm (n = 108). | Not observed | Types B and D; very scarce; between 65 and 105 μm, mean 76 ± 13.9 μm (n = 10) | Only anterior region: Type I; very numerous; between 25 and 45 μm, mean 31 ± 7.7 μm (n = 10). Rest: Type J; very numerous; between 12 and 25 μm, mean 17.2 ± 3.8 μm (n = 10). Type K; scarce to numerous depending on the zone; between 25 and 30 μm, mean 25.3 ± 2.5 μm (n = 10). |
the separation of the lamellae, so that these perform their physiological role more efficiently. Their distribution should be both in the axis and in the lamellae, as in fact occurs in *Cadlina luteomarginata* (Penney, 2006). However, in the studied species most of the spicules are located in the axis of the

Figure 9. Specimen of *Felimare villafranca*, relaxed (A) and contracted (B). Specimen of *Felimida krohni*, relaxed (C) and contracted (D). Scale bars ...
rhinophores (Figure 8B, C) and they are almost absent in the lamellae. Concerning the gills, in general, the number of spicules is low and they are arranged mainly at the base, and in a lower region along the rachis (Figure 10B). Moreover, these organs perform perfectly in other dorid species which lack spicules.

Finally, Jörger et al. (2008) noted for a species of the family Parhedylidae Thiele, 1931 that the aggregation of needle-like spicules between the oral tentacles might give the head additional stability while the animal is moving and digging between sand granules in the interstitial environment, although they also pointed out that the function of these accumulations required further investigations. Nevertheless, the species studied by us, as with other Chromodorididae, are not burrowing.

The presence of spicules in the species of Felimare and Felimida, which have previously been considered to be absent, make a deeper study on the body wall necessary in the remaining genera of this family. This necessity is supported by observations in two other Chromodorididae species: Noumena haliclona, in which Rudman (1984) points out their presence in juveniles, though lacking in adults, and Tysrma delicata, in which spicules were observed in adults (Schrödl & Millen 2001). The study should also include the juvenile stages due to the ontogenetic changes shown in the present work and should be extended to other families considered up to now as spicule-less. An explanation of the non-detection of spicules, though present, may be their low number, small size and, sometimes, deep position within the mantle tissue (Schrödl & Millen 2001). These authors also pointed out that spicules may also have been dissolved in material preserved for a long time.

These data on the presence, distribution, shape and ontogenetic changes of spicules could modify the phylogenetic interpretation of previous studies, such as those of Gosliner & Johnson (1999) and Alejandrino & Valdés (2006), or be useful for future research. Moreover, it would be necessary to carry out studies like those started by Penney (2006) to determine the function of these structures.

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**Editorial responsibility: Christiane Tødt**