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Observations on the ontogenetic and intraspecific changes in the radula of *Polycera aurantiomarginata* García and Bobo, 1984 (Gastropoda: Opistobranchia) from Southern Spain

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SUMMARY: *Polycera aurantiomarginata* García and Bobo 1984 has a stable population in the intertidal area of El Portil beach (Huelva, SW Spain). This fact allowed specimens of different sizes to be collected from March 2001 to December 2003. In this paper, the ontogenetic variations of the radula of *P. aurantiomarginata* are studied. The radulae of 141 specimens were examined, 138 from El Portil and 3 from La Herradura (Granada, SE Spain). Specimens of 1.5-2 mm in length lack the typical radula described for *P. aurantiomarginata*. They have the so called pre-radula whose teeth are different in size and shape from the typical radula of the adults. In the specimens of 3 and 4 mm the pre-radula coexists with the characteristic radula, which is the single structure present in the specimens larger than 4 mm. The following features of the radula are included in this study: radular length, number of teeth rows and length of the outer lateral teeth. According to the three measured variables, the affinities among specimens without a pre-radula were established through cluster analysis, which defined three different groups (4-10 mm, 11-22 mm and 23-48 mm). Correlations between specimen length and radula length, number of rows and mean length of outer lateral teeth were significant. Feeding strategies could be related to the different morphology of the radula established by the Cluster analysis.

Keywords: *Polycera aurantiomarginata,* Opisthobranchia, Gastropoda, radula, ontogenetic variation.

RESUMEN: CAMBIOS ONTOGENÉTICOS E INTRAESPECÍFICOS OBSERVADOS EN LA RÁDULA DE *POLYCERA AURANTIOMARGINATA* GARCÍA AND BOBO, 1984 (GASTROPODA OPISTHOBRANCHIA). – *Polycera aurantiomarginata* García y Bobo, 1984 muestra una población estable y en la localidad de El Portil (Huelva, SW de España), lo que ha permitido la recolección, desde marzo de 2001 hasta diciembre de 2003, de un alto número de ejemplares de todos los tamaños. En el presente estudio se ha extraído la rádula de 141 animales, 138 recogidos en la zona intermareal de El Portil y 3 en La Herradura (Granada). Los tamaños de los animales han oscilado entre 1.5 mm y 48 mm. Se ha podido observar en los individuos de entre 1.5 y 2 mm la existencia de una pre-rádula cuyos dientes son morfológicamente diferentes a los de la rádula de los individuos mayores; sin embargo, en los ejemplares de 3 y 4 mm esta pre-rádula coexiste con la rádula típica, siendo esta estructura la única presente en individuos de longitud igual o mayor a 4 mm. A cada una de las rádulas extraídas, tanto con pre-rádula o sin ella, se le ha medido la longitud total de la cinta, la longitud del diente lateral externo y el número de filas de dientes. Considerando los tres parámetros medidos, las afinidades entre los ejemplares sin pre-rádula se establecieron a partir de análisis de Cluster, que definieron tres grupos distintos (4-10 mm, 11-22 mm y 23-48 mm). Las correlaciones existentes entre la longitud de los individuos y la longitud de la rádula, el número de filas y la longitud media de los dientes fueron significativas. Las diferencias morfológicas reconocidas en los grupos considerados podrían estar relacionadas con distintas estrategias alimentarias.

Palabras clave: *Polycera aurantiomarginata,* Opisthobranchia, Gastropoda, rádula, variación ontogenética.

INTRODUCTION

The morphology of the radular teeth, including aspects such as the number of teeth rows and teeth per half-row, has often been used to differentiate species of opisthobranchs. Nevertheless, the intraspecific and ontogenetic radular variations in the opisthobranchs have been proved in some species. Bleakney (1989) revealed a morphological variation of the radula of *Placida dendritica* (Alder and Hancock, 1843), comparing populations from the Pacific and Atlantic. Bertsch (1976, 1978a, 1978b) studied the radulae of *Discodoris evelinae* Marcus, 1955 and several genera of Chromodorididae, concluding that there is a morphological variation between the newly formed teeth, situated in the newer rows, and the other teeth, situated in older rows. Furthermore, the number of teeth rows increases with the length of the animal.

Pruvot-Fol (1926) described the presence of a "pre-radula" to distinguish the first teeth rows from the remaining rows, during the juvenile stage of the policerid nudibranch *Polycera quadrilineata* (Müller, 1776). Focusing on the family Polyceratidae, Ferreira (1977) reported different radular patterns between juvenile and adults in species of the genus *Triopha* Bergh, 1880. Recently, Ocaña *et al*. (2004) provided data on the ontogenetic variation in number and morphology of radular teeth in several species of genus *Tambja* Burn, 1962, comparing juvenile and adult stages.

This paper examines intraspecific radular variations of *Polycera aurantiomarginata* García and Bobo, 1984 from southern Spain, and provides data on the variation in number and morphology of teeth rows in specimens at different stages, between 1.5 and 48 mm in length.

MATERIAL AND METHODS

The present study was conducted using specimens from El Portil beach (Huelva, SW Spain) (37º12´40´´N, 7º7´50´´W) and La Herradura (Granada, SE Spain) (36º44´N, 3º43´W) (Fig. 1). Three animals were collected in La Herradura and 138 of them in El Portil from March 2001 to December 2003. The specimens were carried alive to the laboratory, where they were measured and fixed in 4% formalin. The radulae of all the specimens were studied by using optic microscopy and those of the juveniles were also photographed using SEM. The length of the radula, the number of rows and the length of the outer lateral tooth were measured with a micrometer ocular. To determine the size of the radular teeth we considered the length of the outer lateral tooth, from the apical cusp to the base (Fig. 2).

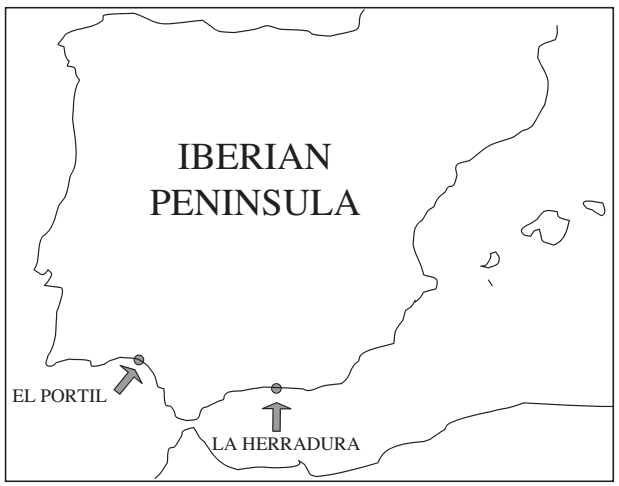

FIG. 1. – Map of Spain showing the sampling locations.

The affinities among specimens according to the three measured variables (radula length, number of rows and the mean values of tooth length) were established through Cluster analysis using the UPGMA method (Unweighted Pair Group Method using Arithmetic averages) (Sneath and Sokal, 1973), based on Euclidean distance. For these analyses the four specimens with a pre-radula were not included. Once the groups of specimens were established through the Cluster analysis, the possible differences of the three

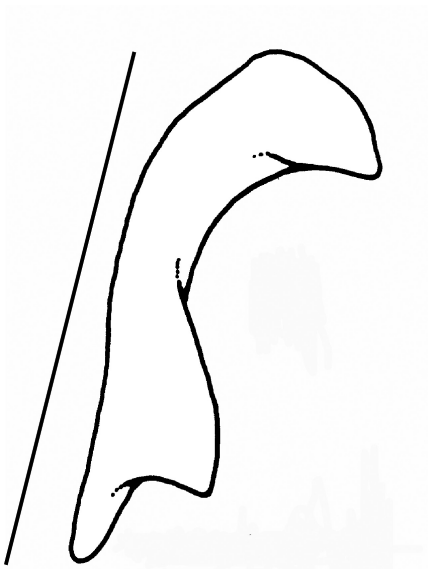

FIG. 2. – Outer lateral tooth. The bar shows the measured length to determine the size of the teeth.

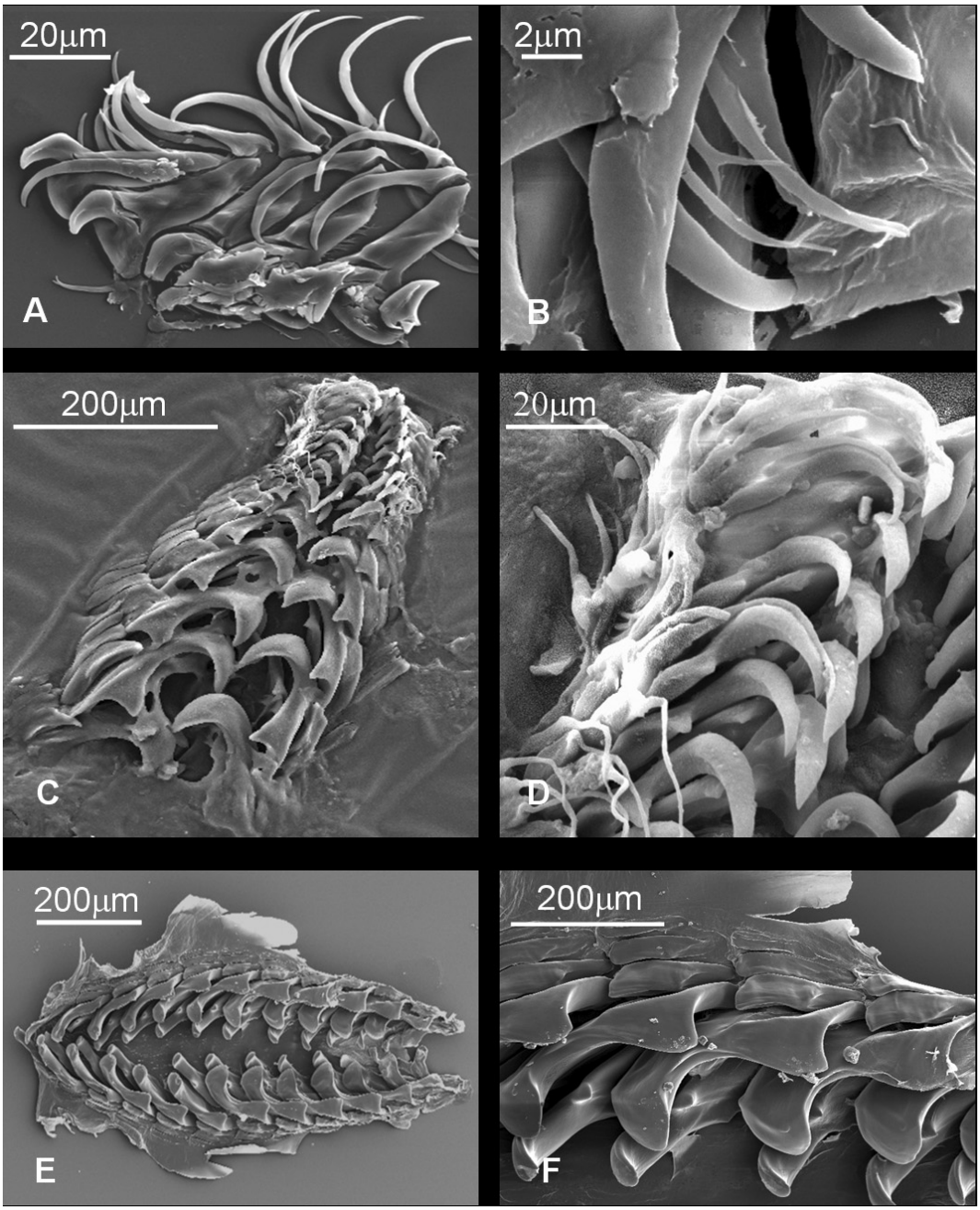

FIG. 3. – SEM photographs of the radula and pre- radula in different specimens of *P.aurantiomarginata*. A. Older portion of the radula in an individual 1.5 mm long. B. Detail of a bifid tooth in the radula of an individual 1.5 mm long. C. Radula and pre-radula of a specimen 4 mm in length. D. Detail of the pre-radula portion from a juvenile 4 mm in length. E-F. Adult radula.

measured variables between groups were tested using the non-parametric Kruskal-Wallis test, after testing the data for normality using the Kolmogorov-Smirnov test and Levene's test for homogeneity of variances. Multivariate analyses (cluster) were carried out using the PRIMER package (Clarke and Gorley, 2001) and for univariate analyses (mean comparison through Kruskal Wallis) the BMDP was used (Dixon, 1983).

RESULTS

Morphological variation of the radular teeth

The radula of *P. aurantiomarginata* is defined as having the formula 8-15 x 4.2.0.2.4 (Figs. 3E, F). The four marginal teeth are quadrangular, without cusps and the size decreases outwards (Fig. 3F; Fig. 4C). The two internal teeth are hamate, as the inner

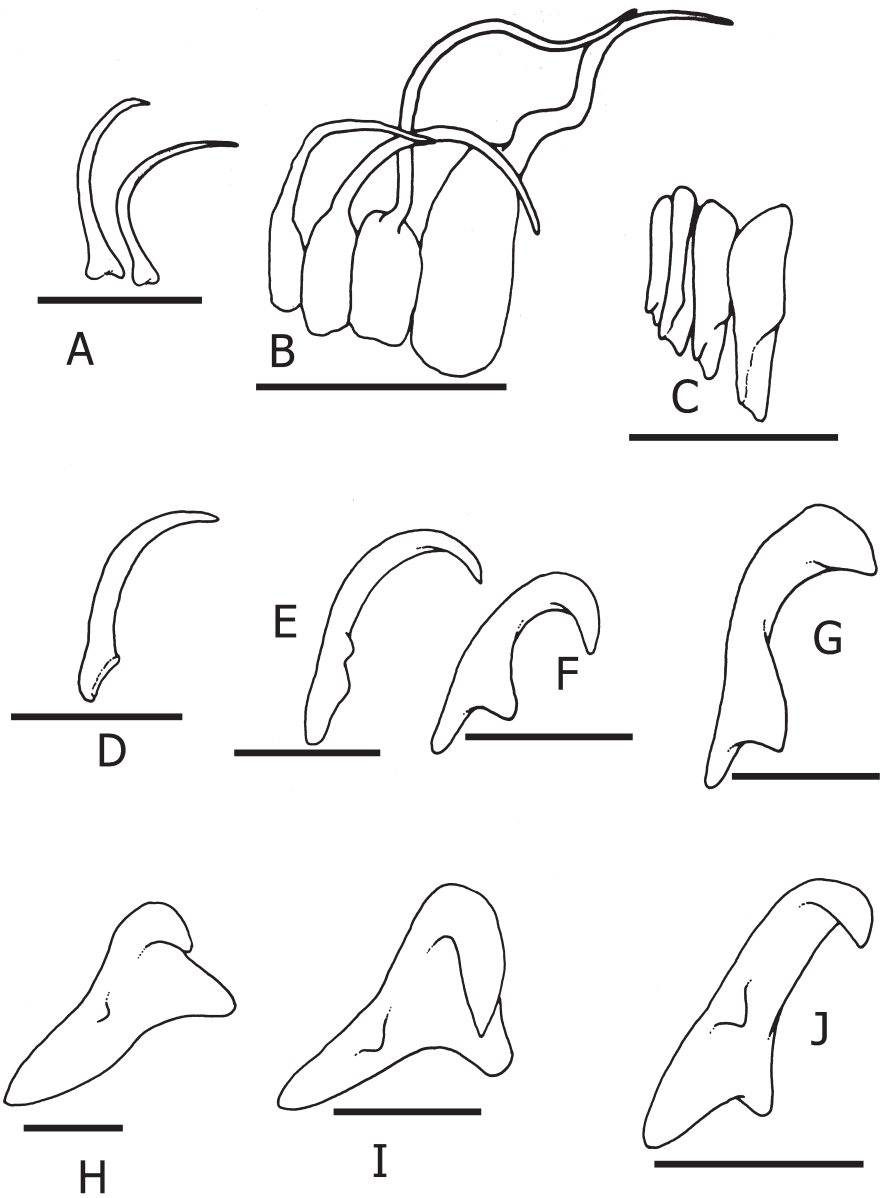

FIG. 4. – Ontogenetic variations in the radula and pre-radula. A, B and C, variation of the marginal teeth in animals smaller than 3 mm (A), 3-4 mm in length (B) and bigger than 4 mm (C). D, E, F and G, variation in the outer lateral tooth in animals smaller than 3 mm (D), 3-4 mm in length in the pre-radula portion (E) and radula portion (F) and individuals bigger than 4 mm (G). H, I and J, variation of the inner lateral tooth in animals smaller than 3 mm (H), 3-4 mm in length (I) and individuals bigger than 4 mm (J). The value of the scale bars are 10mm in H, 20mm in A, B, D, E and I, 100mm in F and 200mm in C, G and J.

tooth is smaller and thinner than the second one; they have an apical cusp and a spur approximately in the middle of the tooth (Figs. 3E, F; Figs. 4G, J). There is no rachidian tooth. This radular pattern, considered as the adult type of the species, appears in almost all the specimens studied. However, some morphological differences are seen in specimens smaller than 4 mm in length.

Specimens of 3-4 mm in length have the radula divided into two portions, with morphological variations of the teeth. The anterior teeth rows, which are the oldest, have the marginal teeth provided by an enlarged basal part and a filamentous cusp (Fig. 3D; Fig. 4B); the lateral teeth have the adult morphology, although the spurs are very small (Figs. 4E, I). The newer rows have the adult pattern described before (Fig. 3C; Figs. 4F, J). In this situation, the anterior portion of the radula is considered as a pre-radula, according to Pruvot-Fol (1926). Specimens smaller than 3 mm lack the adult radular pattern (Fig. 3A), or it only appears in the last rows of the ribbon. The marginal teeth are filamentous, lacking

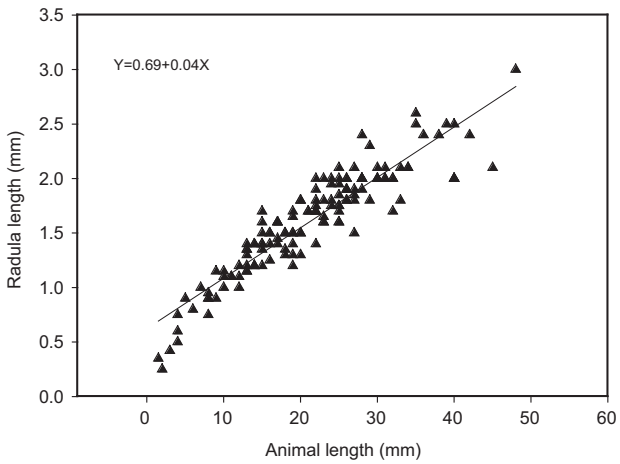

FIG. 5. – Relation between the animal's length and the length of the radula.

the basal enlarged portion; some marginal teeth have the tip bifid (Figs. 3A, B; Fig. 4A). The outer lateral teeth lack the spur and the cusp is provided by a longitudinal furrow (Fig. 4D). The inner lateral teeth have the adult morphology, but the spurs are very small (Fig. 4H).

Relation between the length of the animal and the size of the radula and teeth

Figure 5 shows the relation between the length of the animals and the length of the radula. The relation between the length of the specimens and the mean value length of the teeth is shown in Figure 6. Both graphs show a statistically significant positive correlation. The length of the radula increases with the greater length of the animal (Fig. 5). This line is described by the equation Y = 0.69 + 0.04x, with the coefficient of correlation (r) equal to 0.91 (n = 141, P <0.001). The length of the outer lateral tooth (considered the mean value length of all rows for each specimen) increases as the length of the animal increases (Fig. 6). The regression line is Y = 154.36 + 5.99x; r = 0.92 (n =137, P <0.001).

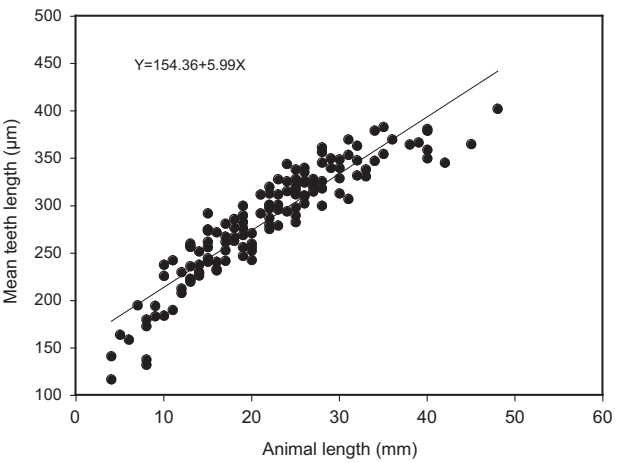

FIG. 6. – Relation between the animal's length and the mean length of the outer lateral tooth.

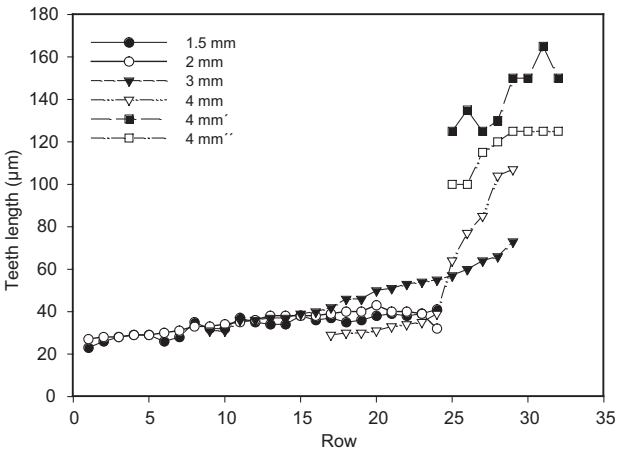

FIG. 7. – Size of the outer lateral tooth in each row of the radula ribbon in specimens up to 4 mm in length. The arrow indicates the beginning of the radula part. Individuals 4 mm in length without a pre-radula are included.

Variation of tooth size along the radula with pre-radula

Besides the ontogenetic morphological variation, the teeth of the pre-radular part differ in size from the adult part. Figure 7 shows the size of the outer lateral teeth along the radular ribbon in specimens up to 4 mm in length. The teeth along the pre-radular rows are notably smaller than the teeth present in adult rows.

Specimens with only pre-radular teeth have all the outer lateral teeth clearly smaller than those animals with adult teeth. In specimens with pre-radular and adult radular rows, the size of the teeth increases quickly from the older rows (pre-radular portion) to the newer rows (with adult teeth pattern). In animals 4 mm in length the pre-radula may be present or absent. When only adult teeth rows are present, the size of the teeth coincides with those of larger specimens.

Radular differences between the number of rows and the length of the specimens

Figure 8 shows the relation between the number of rows and the length of the animals. In this graphic it is possible to see that specimens smaller than 4 mm have more teeth rows than larger specimens.

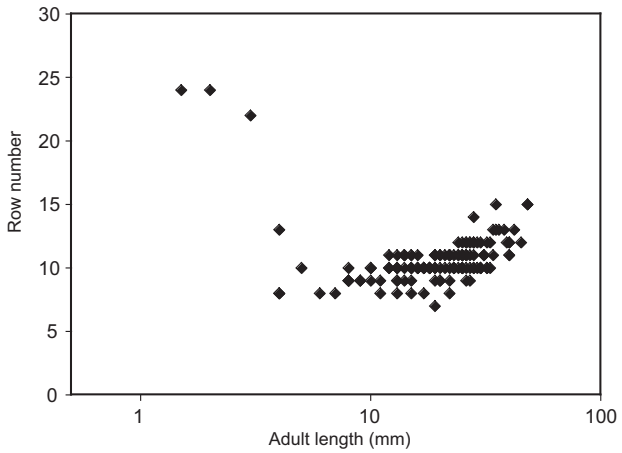

FIG. 8. – Relation between the length of the animal and the number of rows present in the ribbon, the logarithmic scale has been used for the length of the animal.

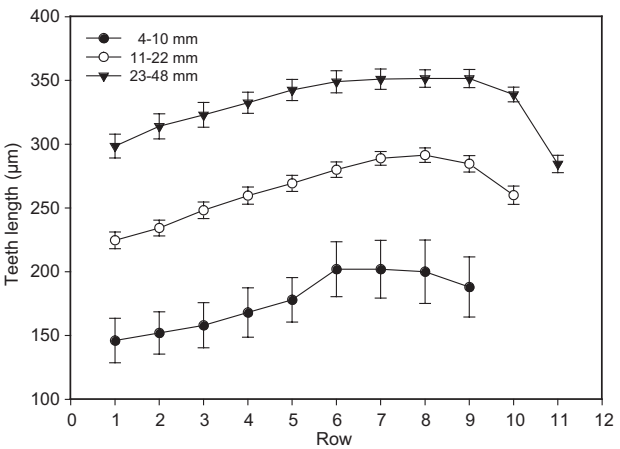

FIG. 10. – Mean values of the outer lateral teeth with the standard error of the mean in each radula row along the ribbon in the three different groups.

There is a significant decrease in the number of rows in specimens larger than 4 mm, and after this, the number of rows increases very slowly with increasing animal size. The sudden decrease coincides with the disappearance of the pre-radula in the animal.

Ontogenetic variation of the radula in *P. aurantiomarginata*

Figure 9 shows the result of clustering the specimens using the UPGMA method and Euclidean distance. The three measured variables (radular length, number of rows and the mean values of tooth length) were used to establish the affinities among specimens. Animals with a pre-radula were not included in this analysis. Cluster analysis distinguished three groups, which represent three animal sizes: 4-10 mm, 11-22 mm and 23-48 mm in length. The non-parametric Kruskal-Wallis test showed statistically significant differences among the three groups for the three measured variables (Table 1).

Figure 10 shows that the mean values of the outer lateral tooth of each radular row reveal that the three groups have different interval sizes, which are larg-

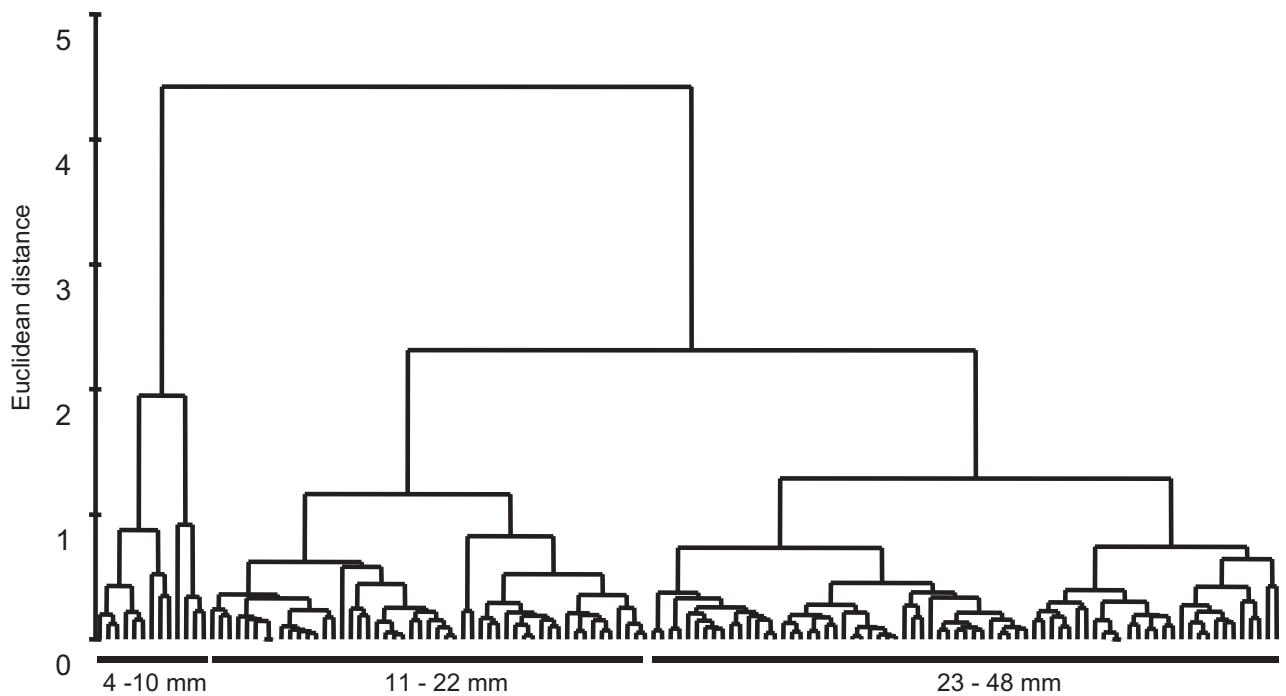

FIG. 9. – The three different groups resulting from the clustering using the UPGMA method and the Euclidean distance. Animals with a pre-radula are not included.

TABLE 1. – Results of the Kruskal Wallis test for the three measured variables: the radula length, number of rows and mean length of the teeth in the three groups established through the Cluster analysis.

	Mean ± Standard deviation			Kruskal Wallis
	4-10 mm	11-22 mm	23-48 mm	(Statistic)
Radula length	0.94 ± 0.16	1.46 ± 0.22	2.00 ± 0.27	805.8***
Number of rows	8.93 ± 0.77	10.00 ± 0.96	11.20 ± 1.27	45.1***
Mean length of the teeth	178.55 ± 36.69	261.49 ± 26.88	333.61 ± 26.08	104.9***

*** p<0.001

er in the animals 23-48 mm in length. However, the size curves along the ribbon are similar for the three groups. Thus, the oldest teeth are smaller because of wear; the size of the teeth increases towards the newer rows, with a maximum length in the sixth to eighth rows. The last rows of the radula (which are the most recently formed) have smaller teeth, which are still developing.

DISCUSSION

Although the opisthobranchs have a certain wide range in the number of teeth per row, this parameter is frequently used as a diagnostic feature at a specific level. Some articles have been published that aim to examine the intraspecific variability related to the radular formula (i.e. Beeman, 1963; Bertsch, 1976; Ferreira, 1977). All of them described a direct correlation between the size of the body and the number of radular rows. However, we have found in *P. aurantiomarginata* that the correlation is not observed if specimens smaller than 4 mm are considered, because in animals up to 3-4 mm the number of radular rows is notably higher than that of specimens larger than 4 mm. Similar situations were described in the species of Polyceratidae *Triopha catalinae* (Cooper, 1863), *T. maculata* MacFarland, 1905, *Tambja ceutae* García-Gómez and Ortea (1988) and *T. marbellensis* Schick and Cervera, 1998 (Ferreira, 1977; Ocaña *et al*., 2004). For both species of *Triopha*, Ferreira (1977) described the presence of a direct function between the size of the adult specimens and the number of rows. However, this author indicated that very small specimens (smaller than 10 mm in length) have a much greater number of rows than the radulae of mature individuals (Ferreira, 1977). In *Tambja ceutae* and *T. marbellensis* the number of radular rows is also higher in specimens smaller than 10 mm long (Ocaña *et al.,* 2004).

Pruvot-Fol (1926) described the presence of the so called pre-radula to distinguish the first teeth rows of *Polycera quadrilineata* from the rest of the rows developed during the juvenile stage. The shape of the teeth of the pre-radula is different from the adult teeth of the radular ribbon. The species of *Tambja* studied by Ocaña *et al*. (2004) also show some changes which coincide with the idea of the presence of a different developmental pattern of the radula in relation to the size (or age) of the specimens. During the development of animals from the juvenile to adult stages the radula changes in the number of rows and the shape of the teeth, independently of the species. This pattern of radula with pre-radula, coincides with that found in *P. aurantiomarginata*. According to the idea of Pruvot-Fol (1926), who describes a pre-radula in *Polycera quadrilineata*, the observations of Ferreira (1977) and Ocaña *et al*. (2004) in the *Triopha*, and *Tambja* species respectively, and in *P. aurantiomarginata* (present paper), it is possible to suppose that in Polyceratidae there is a transition in radular morphology from young to adult animals that takes place rapidly. This variation could be related to a change of diet not confirmed yet by analysis of stomach contents.

Finally, based on our study the following conclusions can be made about *P. aurantiomarginata*:

1) The length of the radula increases with the length of the specimen.

2) The length of outer lateral teeth increases with the length of the animal. Furthermore, the teeth along the pre-radular rows are clearly smaller than adult teeth.

3) Specimens smaller than 4 mm with a pre-radula have more teeth rows than those larger than 4 mm.

4) In specimens without a pre-radula, the number of teeth rows increases as the length of the animal increases.

5) According to the ontogenetic variations observed in the radula of *P. aurantiomarginata*, four groups of specimens are distinguished, which represent different animal sizes: smaller than 4 mm with a pre-radula, 4-10 mm, 11-22 mm and 23-48 mm.

There is no clear reason to explain the cluster separation of the different groups; however, our own observation leads us to think that this separation could be related to feeding strategies or food type. Probably, there is a change in diet in *P. aurantiomarginata* during its growth, which would be related to the type of radular teeth present. The smallest specimens (1.5 to 4 mm in length) were found on the bryozoan *Sessibugula barrosoi* López de la Cuadra and García-Gómez, 1994 (pers. obs.), which leads us to think that this species could be its prey diet. However, the larger specimens (which include the three remaining cluster groups) were found eating the bryozoan *Bugula neritina* (Linnaeus, 1758) and *Tricellaria occidentalis* (Trask, 1857).

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