Tooth microstructure and feeding biology of the brittle star *Ophioplocus januarii* (Echinodermata: Ophiuroidea) from northern Patagonia, Argentina

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Received 28-VII-2014. Corrected 10-X-2014. Accepted 20-XI-2014.

Abstract: *Ophioplocus januarii* is a common brittle star on soft and hard substrates along the Argentinian and Brazilian coasts. Based on stomach contents, tooth microstructure and field observations we identified its food. Opposed to previous suggestions, *O. januarii* appears to be a microphagous species feeding on macroalgal fragments (found in 60.0 % of the analyzed stomachs with content), plant debris (28.0 %), animal cuticle structures (13.0 %), and unidentifiable material (30.7 %). Less frequent items found were foraminiferans, ostracods, an amphipod, a juvenile bivalve, and other crustaceans. Electronic microscope revealed digested material, diatoms and small crustacean appendices. Thus, *O. januarii* is an omnivorous species, feeding mainly on algae, complemented opportunistically with other items. Suspension feeding was observed in the field. It has an fenestrated arrangement intermediate between the previously described uniform and compound teeth. Rev. Biol. Trop. 63 (Suppl. 2): 353-360. Epub 2015 June 01.

Key words: brittle star, stereom microstructure, stomach contents, Ophiolepididae, teeth.

Ophiuroids display diverse feeding strategies, often combined, and ranging from predation and scavenging to deposit- and suspension feeding (Warner, 1982; Harris et al., 2009). Based on the size of the ingested particles, Warner (1982) proposed the division between macrophagous and microphagous species, with the former acquiring large particles through predation and scavenging while the latter include suspension feeding and the acquisition of small particles from the substratum. Some species exhibit high degrees of flexibility in their feeding mechanisms and their prey spectra (Fontaine, 1965; Warner, 1982), and it has been proposed that for both, shallow water and deep-sea species, differences in diets between species also reflect differences in lifestyles. Hereby they may act as non-specialized opportunists with respect to diet (Pearson & Gage, 1984).

Information available on the diet of brittle stars has been established through field observations estimating the feeding preferences by the observed feeding behavior (Warner, 1982), or by stomach content analyses (Harris et al., 2009). Depending on their behavior it is possible to identify active predators or suspension-feeding species (Davoult & Gounin, 1995), while is difficult to directly observe those species that feed on deposited material from the sediment surface or beneath it (Gielazyn et al., 1999). Prey items that are found in stomach content studies allow for the identification of an ophiuroid being microphagous, but also for the general source of the respective food particles: planktonic prey items suggest a filter feeding mode on water-borne items, whereas considerable amounts of sediment may indicate a surface- or subsurface deposit feeding mode (Ferrari & Dearborn, 1989; Dahm, 1999). A third approximation of brittle star feeding preferences is relatively poorly developed and is based on the analysis of the stereom microstructure of the teeth (i.e. how calcite is arranged or disposed in the teeth). Medeiros-Bergen (1996) analyzed the stereom microstructure of teeth from several ophiuroid species using light and scanning electron microscopy. She defined two tooth types: a uniform teeth, in which the tooth stereom is completely fenestrated, and compound teeth, where the base of the tooth is fenestrated, while the distal edge is composed of imperforated calcite. With a few exceptions, macrophagous feeders possess uniform teeth, while microphagous species reveal compound teeth (Medeiros-Bergen, 1996).

The brittle star *Ophioplocus januarii* (Lütken, 1856) (Ophiolepididae) is distributed along the Western Atlantic coast from Golfo Nuevo, Argentina, to northern Brazil, with a doubtful record from Barbados (Thomas, 1975; Bernasconi & D'Agostino, 1977). It is found from the tidal zones down to 74 meters depth, both on hard substrates and on soft sediments rich in detritus (Bartsch, 1982). To date very little is known about the feeding biology of *O. januarii* (Brogger et al., 2013). Bartsch (1982) analyzed stomach contents of some individuals finding only sediment grains. Medeiros-Bergen (1996) observed the microstructure of the teeth of *O. januarii* under a light microscope and described it as completely fenestrated, similar to those teeth she observed in *Ophioplocus esmarki* Lyman, 1874 with the scanning electron microscope.

Here we report on the diet of *O. januarii* and discuss its feeding biology based on stomach content analysis, feeding mechanisms observed in the field, and tooth stereom microstructure analysis using scanning electron microscopy. For comparison, we also describe and discuss the microstructure of the teeth of other brittle stars species and their relations to the respective feeding habits.

MATERIALS AND METHODS

Ophioplocus januarii were collected monthly from Playa Villarino (42º 24' S - 64º 17' W), Golfo San José (northern Patagonia), from April 2005 to April 2007 comprising a total of 25 consecutively taken samples. During each sampling event, the specimens were captured from the same location by SCUBA diving at depths between 2 and 7 m (depending on the tides) and collected in plastic bags. At the laboratory, they were fixed in Bouin's solution for 24 h and then preserved in 70 % ethanol. When individual remaining particles were found in the collection bags they were also preserved in 70% ethanol for later observation. While sampling, brittle stars were observed and photographed in situ in order to identify and determine feeding activities. All sampling and observations were conducted during daylight.

Stomach contents: The discs were cut off along with the stomach lining, and the contents picked out. Stomach contents of 10 brittle stars from each month (total N = 250) were examined under the light microscope, and all content items were separated. When at least one item from a stomach was identified, the respective brittle star was considered 'with stomach content', including unidentifiable and/or digested remains. Only those ophiuroids without any stomach content were considered 'empty'. Some portions of the unidentifiable or digested remains were examined with the light and scanning electronic microscope (SEM).

Tooth microstructure analysis: The jaws of *O. januarii* were dissected and placed for a few minutes in a diluted solution of commercial house bleach in order to remove the epidermal layer. Longer maceration allowed for the dissociation of individual teeth from the dental plates. In order to observe the internal calcite microstructure, one tooth was fractured. For comparative purposes, the jaws and teeth of five other brittle star species were examined as well. These included *Ophiomyxa vivipara*, *Amphipholis squamata*, *Ophiactis asperula*, *Ophiacantha vivipara* and *Ophiocten amitinum*. All jaws and teeth were prepared for SEM observations. At least 7 adult individuals of each species were dissected during the tooth microstructure analysis.

RESULTS

The remaining particles from the individual collection bags resulted to be small shell fragments mixed with algal fragments originating from the sediment. These particles were similar to objects that are sometimes retained or hooked by the ophiuroid's arm spines and other body parts. In no case there were signs of these particles being egested stomach contents from prior to the animals' fixation.

Typical passive suspension-feeding activities were observed in the field. While feeding, the individuals raised two or three arms into the passing currents. Small particles were trapped by the tube feet and collected into a bolus that was passed down along the arm to the mouth.

Stomach contents: Of the 250 individuals examined, 31.2 % had stomachs with contents. Of these, 64.1 % presented only one food item, and far lower percentages referred to stomachs containing two to five different items (Table 1). The most frequent item found corresponded to macroalgal fragments, mainly from filamentous algae. Food particle sizes were up to 0.5 mm with the exception of two larger structures: a macroalgal fragment of 6.0 mm, and a cuticular structure of 7.5 mm. The average number of food items was 1.64 per individual.

Macroalgal fragments were present in 60 % of the stomachs with contents, and in 64 % of the studied months. Other frequent items found were unidentifiable material (31%) and small terrestrial plant debris (28 %). Less frequent items were cuticular animal structures (13%), unidentifiable laminar structures (8 %), spicules (4 %), three foraminiferans, three ostracods, one amphipod, other crustaceans, one juvenile bivalve and one hydrozoarian colony. No sediment particles were found inside the stomachs.

TABLE 1
Total number of stomachs with content and percentage of stomachs with distinguishable amounts of food item

	Number	Percentage
Stomachs analyzed	250	
With contents	78	31.2
With one food item	50	64.1
With two food items	13	16.6
With three food items	9	11.5
With four food items	5	6.4
With five food items	1	1.3

Tooth microstructure analysis: All teeth in *O. januarii* revealed a fenestrated stereom microstructure (Fig. 1). However, it was possible to clearly identify two distinct regions: the basal parts of the teeth which are in contact with the dental plate, are more loosely perforated than the distal parts. There the calcite is much more compacted, presenting a clear different arrangement (Fig. 1 A, B, C, D). This superficial distinction is visible also internally in the calcite microstructure as can be seen in a fractured tooth (Fig. 1 F, G). The basal surface presents little serrated edges, but at the distal portion these edges are less prominent because of the tightly packed calcite (Fig. 1 E).

Under light microscope, the teeth of *Ophiomyxa vivipara* presented translucent crystalline edges with small spines protruding from the distal edges; they are semicircle in shape and are perforated apart from the distally protruding spines (Fig. 2 A). The teeth of *Ophiacantha vivipara* and *Ophiocten amitinum* presented spine like shapes with uniform fenestrated surfaces and sharply serrated edges (Fig. 2 D, E). *Amphipholis squamata* carried teeth with a fenestrated base, but distally the calcite was imperforate (Fig. 2 B). The tooth stereom microstructure of *Ophiactis asperula* is similar to that described here for *O. januarii*, and consisted of two regions with differentiated calcite compactions (Fig. 2 C). The tooth types of the

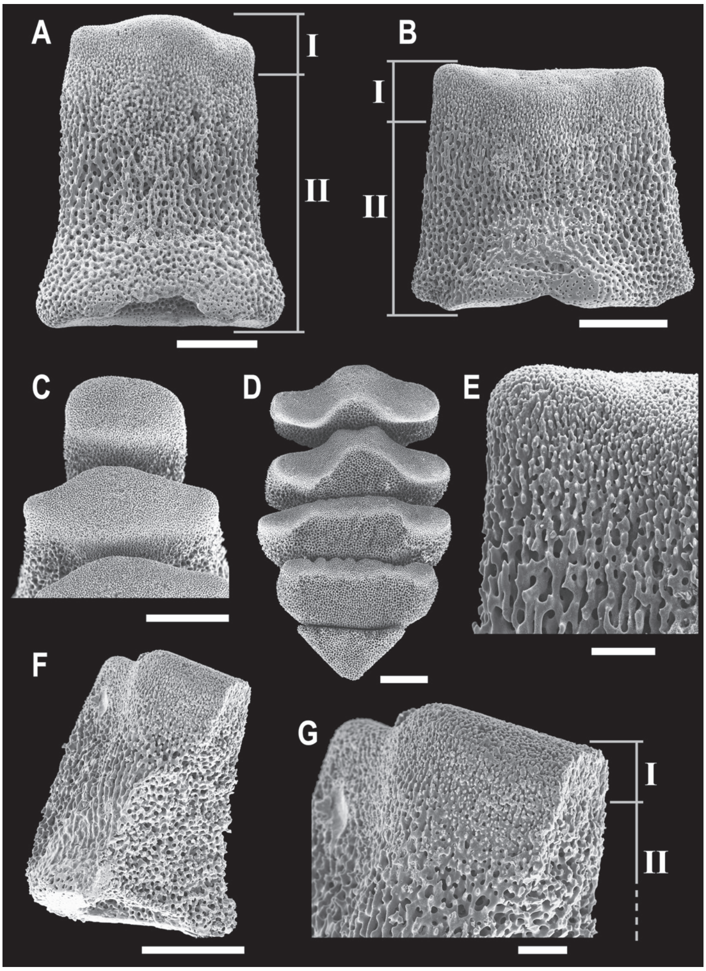

Fig. 1. Scanning electron microscopy images of teeth in *Ophioplocus januarii*. A) Dorsalmost tooth. B) Mid-positioned tooth. C) Proximal view of dorsal teeth. D) Proximal view of ventral teeth. E) Detail of B, showing the different calcite compaction. F) Fractured tooth. G) Detail of F, showing the internal differentiation in microstructure. I and II indicate regions of more compaction (I) and more porosity (II) of the calcite, respectively. Scale bars: A, B, C, D = 200 µm; E, G = 50 µm.

six species analyzed presently are summarized in the Table 2.

DISCUSSION

In the present study, *Ophioplocus januarii* from Playa Villarino fed ingesting small-suspended particles through suspension feeding from the water-sediment interface. Contrarily to previously made assumptions (Warner, 1982; Medeiros-Bergen, 1996), *O. januarii* is a microphagous species. It fed opportunistically, mainly ingesting fragments of macroalgae, but also small plant and animal structures, and other suspended material. When analyzing stomach contents from a 60 meters depth *O. januarii* population collected in a nearby location on the continental shelf (42° S - 62° W), Bartsch (1982) observed stomachs lacking food but filled partly with sediment grains. In the present study, no sediments were found, suggesting differences in feeding preferences to be

Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744) Vol. 63 (Suppl. 2): 353-360, June 2015

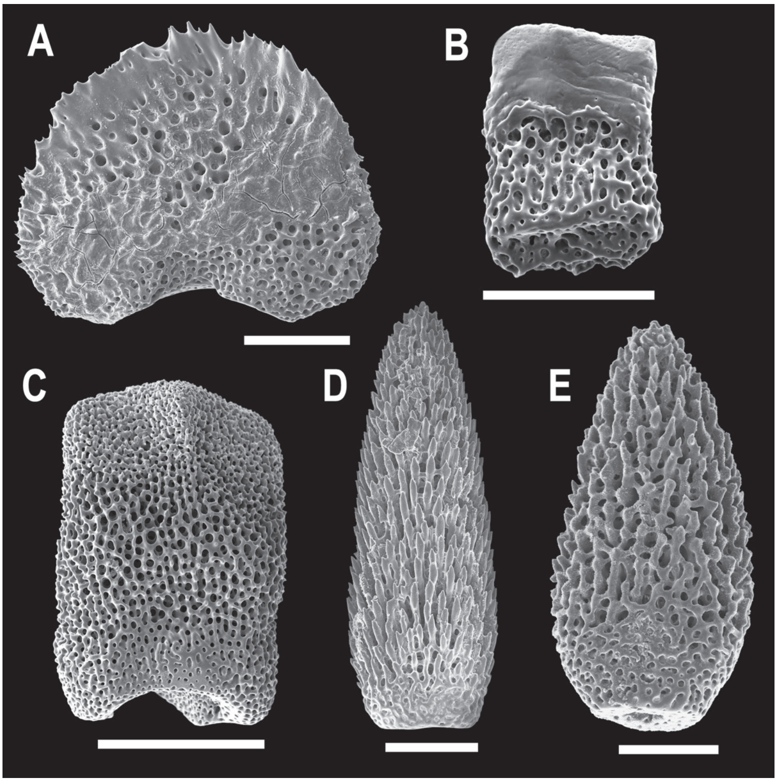

Fig. 2. Scanning electron microscopy images of tooth stereom microstructure in different ophiuroid species (mid-positioned teeth). A) *Ophiomyxa vivipara*. B) *Amphipholis squamata*. C) *Ophiactis asperula*. D) *Ophiacantha vivipara*. E) *Ophiocten amitinum*. Scale bars: A, C, D = 200 µm; B, E = 100 µm.

TABLE 2
Tooth stereom microstructure for six analyzed ophiuroid species: uniform (completely fenestrated), compound (fenestrated base and imperforate calcite tip) and intermediate (fenestrated with two distinct regions) tooth types

Species	Family	Source	Tooth type
Ophioplocus januarii (Lütken, 1856)	Ophiolepididae	42º 24' S; 64º 17' W (2 - 7 m)	intermediate
Amphipholis squamata (Delle Chiaje, 1828)	Amphiuridae	44º 53 'S; 65 º40' W (0 m)	compound
Ophiacantha vivipara Ljungman, 1870	Ophiacanthidae	38º 51' S; 55º 39' W (115 m)	uniform
Ophiactis asperula (Philippi, 1858)	Ophiactidae	38º 51' S; 55º 35' W (145 m)	intermediate
Ophiocten amitinum Lyman, 1878	Ophiuridae	38º 51' S; 55º 35' W (145 m)	uniform
Ophiomyxa vivipara Studer, 1876	Ophiomyxidae	43º 36' S; 60º 05' W (96 m)	uniform

dependent of habitat diversity. This was also observed for different populations of *Ophiura ophiura* (Blegvad, 1914 in Warner, 1982) and of *Ophionotus victoriae* (Dearborn, 1977). The main distinction between feeding types in brittle stars used to be drawn between carnivory and microphagy (Warner, 1982). Those species that capture large particles –typically of animal origin– and, thus, feed as microphagous feeders, are generally described as carnivorous, while microphagous species feed on a mixture of minute animal and vegetal material. The diet presently observed for *O. januarii* indicates that this species is an unselective omnivorous species. Others also feeding mainly on vegetal origin material are mostly associated with sediments. *Ophionereis reticulata*, for example, has been indicated as exclusively herbivorous

(May, 1925 in Warner, 1982) and more recently as an omnivore with algal feeding preference (Yokoyama & Amaral, 2008). Algal and calcareous fragments are the most frequent items in the diets of *Ophiocoma wenditii*, *O. echinata* and *O. pumila* (Sides & Woodley, 1985).

In stomach contents analyses conducted with different species, the percentages of empty stomachs found were highly variable between the species. For example, Harris et al. (2009) observed 66 % of empty stomachs in *Ophiura sarsii*, Yokoyama and Amaral (2008) 23 % in *Ophionereis reticulata*, Hendler (1982) found variations from 100 to 5% with a dependence of the months under study, and Hendler and Miller (1984) observed differences in percentage for *Asteroporpa annulata* according to whether the individuals were captured during day or nighttime. In the present study, the percentage of empty stomachs in *O. januarii* was close to 70 % and included only samples that were collected during the daylight. The fact that brittle stars may egest their stomach contents in response to collecting procedure or handling (Pearson & Gage, 1984; Hendler, pers. comm.), could explain the high proportions of empty stomach found in some deep-sea investigations (Warner, 1982). This, however, does not seem to be the case in the presently investigated *O. januarii* because no egested material was found when analyzing the collection bags.

The tooth stereom microstructure in *O. januarii* is distinctly different from that in teeth of macrophagous as well as microphagous species (Fig. 1; Table 2). The presently found intermediate fenestrated arrangement of the stereom was also here observed for *Ophiactis asperula*, and confirmed as such when analyzing the teeth with the scanning electron microscope (SEM). The fact that SEM reveals structures which might remain disguised under the light microscope could explain that Medeiros-Bergen (1996) did not recognize differences in the tooth microstructure between *Ophioplocus esmarki* and *O. januarii*. Therefore, it is possible that other species previously described as carrying uniform teeth (macrophagous) could, in fact, possess intermediate tooth types. In the present study we identified the intermediate tooth type, and the previously described uniform and compound types. However, it would be interesting to analyze additional species in order to recognize other possible variations in the tooth stereom microstructure previously overlooked.

The present results observed for the diet of *O. januarii* represent, to our knowledge, the first trustworthy report of microphagy in the family Ophiolepididae. Dietary studies on *Ophiolepis elegans* suggested this species to be a macrophagous species (Warner, 1982), while Medeiros-Bergen (1996), based on tooth microstructure, estimated three *Ophioplocus* species and *Ophiolepis impressa* to be microphagous as well. Two other species from the same family (*Ophioplocus incipiens* and *Ophiomusium lymani*) are reported to conduct suspension-feeding activities (Warner, 1982), and little information about their diets is available. Pearson and Gage (1984) suggested *O. lymani* to have an omnivorous diet. Because there are no records of passive suspension feeding in macrophagous species, it is likely that both *O. incipiens* and *O. lymani* being representatives of the Ophiolepididae, are microphagous as well.

Much progress on the knowledge of the diet of ophiuroids has been made since Warner's summary in 1982. Yet, much is still obscure. For example, the role that brittle stars play in trophic transfers of nutrients within benthic communities and between benthic and pelagic communities still remains highly unknown (Gielazyn et al., 1999). The utility of analyzing teeth microstructure may surpass not only knowledge on feeding habits and diet in ophiuroids, but may also provide a helpful tool for phylogenetic issues, most likely in combination with tooth macrostructure analyzes as proposed by Stöhr (2005), and with studies on jaw morphology already used by Murakami (1963), Smith et al. (1995) and Stöhr and Muths (2010).

ACKNOWLEDGMENTS

Thanks to the people from the Laboratorio de Ecosistemas Costeros (MACN) and the LARBIM (CENPAT) for their assistance during collection and processing at the laboratory. This work was funded by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP-0253) and from the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2012-0561 and PICT 2013-2504). We also thank three referees for valuable suggestions, particularly to Karin Boos for her helpful commentaries and recommendations that improved considerably the final manuscript.

RESUMEN

Microestructura dental y biología alimentaria del *Ophioplocus januarii* **(Echinodermata: Ophiuroidea) del norte de Patagonia, Argentina**. El ofiuroideo *Ophioplocus januarii* se distribuye a lo largo de las costas de Argentina y Brasil, encontrándose tanto en substratos duros como blandos. En base al análisis de contenidos estomacales y la microestructura de los dientes, junto a observaciones de campo, se describe el comportamiento alimentario de esta especie. Opuesto a suposiciones previas, *O. januarii* es una especie micrófaga que se alimenta de fragmentos de macroalgas (encontrados en el 60.0 % de los estómagos analizados que presentaban contenido), detritos vegetales (28.0 %), estructuras cuticulares animales (13.0 %) y material inidentificable (30.7 %). Menos frecuente, se encontraron foraminíferos, ostrácodos, un anfípodo, un bivalvo juvenil y otros crustáceos. Pequeñas porciones del material inidentificable fueron analizadas en el microscopio electrónico de barrido, resultando ser material digerido, diatomeas y pequeños apéndices de crustáceos. Así, *O. januarii* es una especie omnívora, que se alimenta principalmente de algas, complementando su dieta de manera oportunista con otros ítems. Las observaciones de campo revelaron alimentación suspensívora. El análisis de la microestructura del estereoma del diente resultó en un arreglo del tipo fenestrado intermedio, que se encuentra entre los dos tipos de arreglos descriptos hasta ahora, los dientes de tipo uniforme y los compuestos. De estos últimos, el primero ha sido encontrado en especies macrófagas mientras que el segundo se corresponde a ofiuroideos micrófagos. En el presente trabajo, se propone la existencia de un nuevo tipo de arreglo intermedio en la matriz dental de los ofiuroideos.

Palabras claves: Estrellas quebradizas, microestructura estereoma, contenido estomacal, Ophiolepididae, diente.

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