

Feeding Behavior of *Spurilla* sp. (Mollusca: Opisthobranchia) with a Description of the Kleptocnidae Sequestered from Its Sea Anemone Prey

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Agustín Garese, Stella García-Matucheski, Fabián H. Acuña, and Claudia Muniain (2012) Feeding behavior of *Spurilla* sp. (Mollusca: Opisthobranchia) with a description of the kleptocnidae sequestered from its sea anemone prey. *Zoological Studies* 51(7): 905-912. Cnidocysts are sequestered from cnidarian prey by aeolid nudibranchs and stored in the tips of their appendages (cerata). The kleptocnidae of 11 specimens of *Spurilla* sp. are described in detail. The types and relative abundances of prey cnidocysts in nudibranch cerata were highly variable, suggesting exclusive anemone consumption. Spirocysts and several types of microbasic p-mastigophores, microbasic b-mastigophores, and basitrichs were found in external and internal tissues of sea anemone prey. This is the 1st report of predation of aeolid *Spurilla* sp. on the sea anemones *Antholoba achates*, *Metridium senile lobatum*, and *Parabunodactis imperfecta* from the rocky intertidal of Patagonia (Chubut Province, Argentina) and on *Anthothoe chilensis* and *Tricnidactis errans* at Mar del Plata (Buenos Aires Province, Argentina). Photographs and digital videos of aeolidacean feeding behavior were recorded *in situ* and in aquaria. The purpose of this study was to provide valuable information on the diet of *Spurilla* sp. from Argentina using field observations and descriptions of the kleptocnidae.
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Key words: Nudibranchia Aeolidiidae, Cerata, Sea anemone, Argentina.

Nudibranchs are shell-less gastropods. Their diverse color patterns, high diet specificity, and mechanical and chemical defensive attributes have attracted the attention of marine biologists and biotechnologists (Su et al. 2009). Aeolid nudibranchs are known to feed upon cnidarians and are capable of sequestering the cnidocysts of their prey. The cnidocysts are ingested, pass through the digestive diverticula, migrate to the tips of the dorsal cerata, and are stored in a functional state in cnidosacs within specialized cells termed cnidophages (Grosvenor 1903, Conklin and Mariscal 1977, Greenwood and Mariscal 1984a, Greenwood 1988). The set of cnidocysts selected by the aeolid is termed kleptocnidae (Greenwood

2009). Some authors also suggested that nudibranchs incorporate immature cnidocysts, which can complete maturation within the cnidosacs (Neville 1926, Greenwood and Mariscal 1984b, Harris 1971 1973).

Cnidarians have different types of cnidocysts, which form the cnidom (assemblage of cnidocysts present in any cnidarian), and vary among species even within their tissues (England 1991, Östman 2000, Fautin 2009). Many aeolid species are specific in the selection of cnidocysts, and the types can vary according to the prey consumed, which provides information about the feeding history of the nudibranch (Day and Harris 1978). Specific types of cnidocysts stored by nudibranchs

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are poorly known, probably due to difficulties with cnidocyst identification (Conklin and Mariscal 1977). Kleptocnidae were often supposed to be defensive (Edmunds 1966, Harris 1973, Aguado and Marín 2007), but direct observational and experimental evidence does not fully support that (Miller and Byrne 2000, Edmunds 2009, Penney et al. 2010).

Spurilla sp. is an intertidal nudibranch recorded for the 1st time in Argentina by Muniain (1997 2004); its distribution range extends from Mar del Plata (Buenos Aires) to Chubut (Patagonia). Preliminary studies on kleptocnidae of *Spurilla* sp. collected from Mar del Plata (Buenos Aires) showed that this species incorporates cnidocysts of the sea anemones *Anthothoe chilensis* (Lesson, 1830) and *Tricnidactis errans* De Oliveira Pires, 1987 (Garese et al. 2009). The present work focused on the diet of *Spurilla* sp. from Argentina using field observations and kleptocnidae examination.

MATERIALS AND METHODS

Nudibranch feeding behavior

Field observations to identify species of sea anemone prey of *Spurilla* sp. were made in the intertidal of Larralde and Punta Gales (Chubut Province), Punta Colorada (Río Negro Province), Punta Cantera, and La Estafeta (Mar del Plata, Buenos Aires Province); all locations are in Argentina. Aquarium experiments were conducted to study in detail the predation steps of the aeolids on sea anemones from Chubut. *Spurilla* sp. specimens together with their prey were collected by hand from intertidal rocks and transferred to the laboratory in containers with natural seawater under controlled conditions of temperature, aeration, and salinity. Sea anemones were first placed in the center of the aquaria; after 2 h of acclimation, groups of 4 nudibranchs were introduced, 1 group at a time, into 3 aquaria, each one containing specimens of the sea anemone species. Observations lasted 3 h and allowed us to describe in detail the sequence of the nudibranch attack, its preference for a particular structure of the sea anemone, and the behavioral response of the prey. After the experiments, specimens were preserved in 75% ethanol for taxonomic studies. Photographs and digital videos of nudibranchs and their prey were taken *in situ* and in aquaria.

Study of the kleptocnidae

In total, 11 specimens of nudibranchs of *Spurilla* sp. were collected by hand from 4 rocky intertidal sites; Punta Gales, Larralde (Chubut Province), Punta Cantera, and La Estafeta (Mar del Plata, Buenos Aires Province). Three specimens were collected from each site, except from Larralde, where only 2 were collected. Specimens were fixed in 5% formaldehyde and subsequently transferred to ethanol. In the laboratory, cerata (Fig. 1A) were haphazardly removed from the animal, using fine-pointed forceps; ceras squashes were then prepared to study the kleptocnidae. At least 3 cerata per individual were analyzed, and at most 100 cnidocysts per ceras were identified and measured using a Zeiss Axiolab microscope with oil immersion at 1000x magnification. When the intended number of cnidocysts had not been reached, more cerata (up to 10) were dissected until we had sampled 300 cnidocysts per individual. In total, 3247 capsules were identified and measured. Cnidocysts were classified according to England (1991). Statistically descriptive parameters, such as the size (length and width) and abundance of the different types of cnidocysts, were obtained using the R program (R 2008). Finally, with those results and making comparisons with available information about the cnidae of common sea anemone species from the sampled sites, we identified probable prey sources of the kleptocnidae found and the specific structure of the prey consumed by the aeolids.

Voucher specimens were preserved at the invertebrate collection (MACN-In) of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires). *Spurilla* sp., 1 specimen, 20 Jan. 2006, MACN-In: 37723, Larralde (42°24'14"S, 64°18'19"W, Chubut Province); 1 specimen, 12 June 2009, MACN-In: 39307, La Estafeta, Mar del Plata (38°15'S, 57°56'W, Buenos Aires Province).

RESULTS

Nudibranch feeding behavior

Specimens of *Spurilla* sp. were observed *in situ* preying on *Antholoba achates* (Drayton in Dana, 1846) at Punta Colorada (Río Negro) (Fig. 1B), *Parabunodactis imperfecta* Zamponi & Acuña, 1992 at Larralde (Chubut) (Fig. 1C), and on patches of *A. chilensis* and *T. errans* at Punta

Cantera (Mar del Plata) (Fig. 1D).

Aeolids were found in rocky intertidal tide pools, most of the time behind rocks in darkness, where sea anemones were common. Laboratory feeding experiments confirmed predation of *Spurilla* sp. on *A. achates* (Fig. 1E) and *P. imperfecta* (Fig. 1F). After an acclimation period, the sea anemones had become tightly attached to the substrate. When we introduced aeolids into the aquaria, they moved immediately towards the prey. Initial contact involved the oral tentacles and rhinophores of the nudibranch with the prey pedal disc. The nudibranchs extended their buccal mass and attached it to the sea anemone to begin feeding. Meanwhile, the sea anemone

contracted its column, withdrew its tentacles inside, and began to detach its pedal disc from the substrate. The nudibranch finished feeding at the top of the sea anemone column, without touching or ingesting the tentacles. We observed that several nudibranchs fed together on the same sea anemone, and similar feeding behaviors were observed by individuals in the 3 aquaria.

Study of the kleptocnidae

Specimens of *Spurilla* sp. from Punta Gales (Chubut) had 8 types of cnidocysts. Specimen 2 showed the most diverse kleptocnidae (6 types), followed by specimen 3 (5 types) and specimen

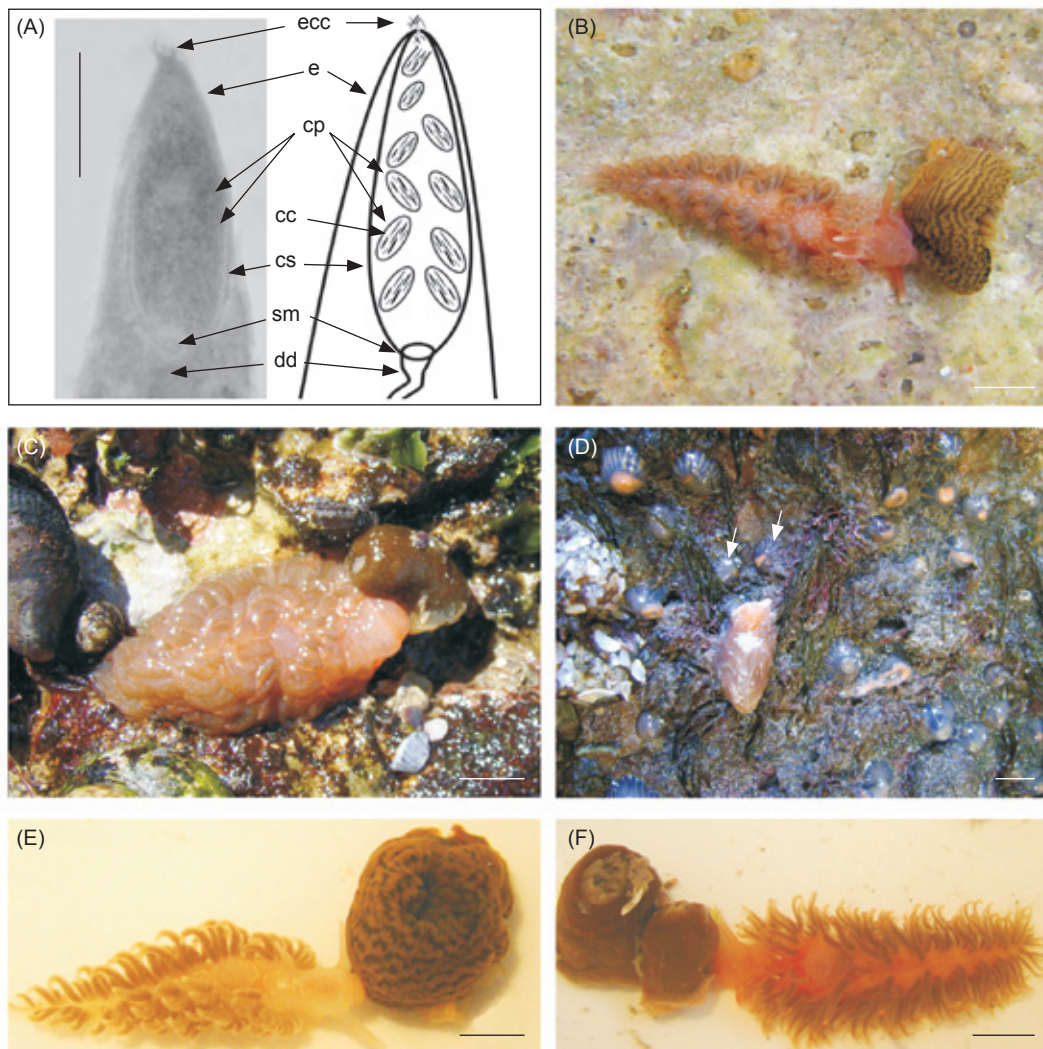


Fig. 1. (A) Cerata structure of *Spurilla* sp. (ecc = expelled cnidocysts, e = epithelium, cp = cnidophage, cs = cnidosac, cc = cnidocysts, sm = sphincter muscle, dd = digestive diverticulum); (B-F), Specimens of *Spurilla* sp. preying. *In situ*: (B), on *A. achates*; (C), on *P. imperfecta*; (D), on *A. chilensis* (arrows). In the laboratory: (E), on *A. achates*; (F), on *P. imperfecta*. Scale bars: A = 1 mm; (B-E) = 1 cm. (Photos: Garese: a,d; G. Matucheski b-f).

1 (3 types). The spirocyst was the only type that appeared in all 3 specimens. The p-mastigophores II and III and an unidentified cnidocyst were only present in specimen 3. Spirocysts were clearly the dominant type in specimens 1 and 2, whereas basitrichs prevailed in specimen 3 (Table 1).

Specimens from Larralde (Chubut) showed 7 types of cnidocysts. Specimen 1 had a low number of cnidocysts per cerata; therefore, it was necessary to analyze a large number of cerates to obtain at least 247 cnidocysts. Basitrich I was the most abundant cnidocyst type, followed by microbasic b-mastigophore II and an unidentified type. Kleptocnidae of specimen 2 were dominated by b-mastigophore types I and II. According to the cnidocyst types together with data on their size ranges identified from the literature (Riemann-Zürneck 1975, Fautin 1984, Zamponi and Acuña 1992, Laretta et al. 2009), there were 3 possible

types of source prey of *Spurilla* sp. in Chubut: *A. achates*, *Metridium senile lobatum* (Carlgren, 1899), and *P. imperfecta*. The most abundant cnidocysts found in most specimens from Chubut corresponded to external structures of the sea anemones (tentacles, column, pseudoacroraghi, and acontia, an inner structure that sea anemones eject when disturbed). Although cnidocysts of internal mesenterial filaments and the actinopharynx were also present, they were usually less abundant (see Table 1).

Specimens from Punta Cantera (Mar del Plata) showed 7 types of cnidocysts, with specimens 1 and 3 each having 6 types and specimen 2 having all types. There was no predominant cnidocyst in specimens 1 or 2. In contrast, for specimen 3, microbasic p-mastigophore I was the most abundant type, followed by basitrich II. Specimens from Estafeta

Table 1. Kleptocnidae of *Spurilla* sp. from Patagonia (Chubut). Cnidocyst type, size, abundance, and probable source prey are indicated

S	Collection site/Cnidocyst type	Range (µm)		n	NC	Abundance (%)	Prey source species and structure
		Length (mean ± S.D.)	× width (mean)				
Patagonia: Punta Gales (Chubut)							
1	spirocyst	15-29 (22.23 ± 2.03)	× 2-5 (3.23)	284	3/3	94.66	Aa (T), Pi (T)
	microbasic b-mastigophore I	15-29 (22.4 ± 4.14)	× 2-4 (3.16)	10	3/3	3.33	-
	microbasic p-mastigophore I	17-23 (20.5 ± 2.16)	× 3-6 (4.75)	6	1/3	2	Pi (Mf),
2	spirocyst	15-27 (19.65 ± 2.22)	× 3-5 (3.4)	184	3/3	61.33	Aa (T), Pi (T)
	microbasic b-mastigophore I	17-21 (19 ± 2.82)	× 4 (4)	2	2/3	0.66	-
	basitrichs I	12-24 (20.55 ± 2.55)	× 2-3 (2.42)	74	3/3	24.66	Aa (T), Pi (C, T),
	basitrichs II	26-45 (33.1 ± 5.9)	× 3-4 (3.27)	28	3/3	9.33	Aa (Ax, T), Pi (P)
	microbasic p-mastigophore I	14-21 (17.54 ± 2.38)	× 4-5 (4.62)	11	3/3	3.66	Pi (Mf),
	microbasic p-mastigophore II	25 × 9		1	1/3	0.33	-
3	spirocyst	13-23 (17.72 ± 2.13)	× 2-5 (3.27)	29	3/3	9.66	Aa (T),
	microbasic p-mastigophore (?)	34-37 (35.6 ± 1.14)	× 5-7 (5.8)	5	1/3	1.66	Ms (A)
	basitrichs I	9-26 (16.05 ± 3.68)	× 2-3 (2.5)	204	3/3	68	Aa (T, C), Pi (C, T, Mf)
	basitrichs II	30-50 (38.2 ± 4.98)	× 3-4 (3.18)	25	3/3	8.33	Pi (P)
	microbasic p-mastigophore III	19-28 (22.27 ± 2.34)	× 5-8 (5.76)	37	3/3	12.33	Aa (Mf, Ax)
Patagonia: Larralde (Chubut)							
1 ^a	spirocyst	17 × 3		1	1/10	0.4	-
	microbasic p-mastigophore (?)	26-63 (41.01 ± 7.88)	× 4-7 (5.44)	59	6/10	23.88	Ms (A)
	microbasic b-mastigophore I	16-21 (22.78 ± 3.67)	× 3-5 (3.9)	28	4/10	11.33	Pi (Mf)
	microbasic b-mastigophore II	12-21 (15.7 ± 2.36)	× 3-5 (3.52)	61	7/10	24.69	-
	basitrichs I	10-25 (16.7 ± 3.26)	× 2-4 (3)	88	6/10	35.62	Aa (T,C), Pi (C, T, Mf)
	basitrichs II	20-28 (25.2 ± 3.03)	× 3-4 (3.4)	5	3/10	2.02	Pi (Ax)
	microbasic p-mastigophore I	17-20 (17.8 ± 1.3)	× 5-6 (5.66)	5	3/10	2.02	Pi (Ax)
2	microbasic p-mastigophore (?)	31-49 (36.81 ± 3.84)	× 5-7 (5.81)	33	4/4	11	Ms (A)
	microbasic b-mastigophore I	17-31 (20.32 ± 3.04)	× 3-5 (3.84)	131	4/4	43.66	Pi (Mf)
	microbasic b-mastigophore II	11-17 (14.25 ± 1.44)	× 3-4 (3.39)	129	4/4	43	-
	basitrichs I	17-27 (22.28 ± 3.72)	× 2-4 (3)	7	4/4	2.33	Aa (T, Mf)

S, specimen number; n, number of capsules measured; NC, proportion of cerates containing cnidae. Species: Aa, *Antholoba achates*; Ms, *Metridium senile lobatum*; Pi, *Parabunodactis imperfecta*. Sea anemone structures: A, acontia; Ax, actinopharynx; C, column; Mf, mesenterial filament; P, pseudoacroraghi; T, tentacles. ^a n < 300 due to the scarce number of cnidocyst in the cerates.

(Mar del Plata) showed 4 types of cnidocysts, with the type p-mastigophore I being dominant among the 3 aeolids. Specimen 3 was the only one that had the p-mastigophore type III, although only 1 capsule was found. The kleptocnidae isolated from individuals from Punta Cantera were more diverse than those from Estafeta. Cnidocyst types together with data on size ranges from the literature (Pires 1988, Excoffon et al. 1997) allowed us to identify 2 probable types of source prey: *A. chilensis* and *T. errans* at Mar del Plata. In these specimens, we observed a high abundance of different cnidocysts, although in some cases, the microbasic p-mastigophore I was particularly abundant. These types belonged to both internal

and external tissues of the sea anemones (Table 2).

DISCUSSION

Our results, based on a study of the kleptocnidae and field observations, showed that *Spurilla* sp. feeds exclusively on sea anemones at various intertidal localities from Buenos Aires Province to Patagonia. Specifically, these aeolids prey on the species *A. achates*, *M. senile lobatum*, and *P. imperfecta* at Chubut, on *A. achates* at Río Negro, and on *A. chilensis* and *T. errans* at Mar del Plata (Buenos Aires).

Feeding experiments showed that *Spurilla* sp.

Table 2. Kleptocnidae of *Spurilla* sp. from Mar del Plata (Buenos Aires). Cnidocyst type, size, abundance, and probable prey sources are indicated

S	Collection site/Cnidocyst type	Range (µm) Length (mean ± SD) × width (mean)	n	NC	Abundance (%)	Prey source species and structure
Mar del Plata: Punta Cantera (Buenos Aires)						
1	spirocyst	16-26 (21.15 ± 2.78) × 2-5 (3.5)	38	3/3	12.66	Te (T), Ac (T)
	microbasic b-mastigophore	13-23 (18.76 ± 2.71) × 2-5 (3.9)	68	2/3	22.66	Te (Ax)
	basitrichs I	13-31 (25 ± 4.61) × 2-3 (2.25)	67	3/3	22.66	Te (A), Ac (T, Ax)
	basitrichs II	26-30 (28.14 ± 1.57) × 2-3 (2.27)	7	1/3	2.33	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	14-24 (19.12 ± 2.57) × 3-5 (4.07)	58	2/3	19.33	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	42-71 (58.9 ± 6.65) × 5-8 (6.62)	62	3/3	20.66	Te (A), Ac (A)
2	spirocyst	12-19 (14.83 ± 2) × 3-5 (3.68)	18	4/6	6	Te (T), Ac (T)
	microbasic b-mastigophore	14-22 (17.27 ± 2.09) × 3-4 (3.68)	61	6/6	20.33	Te (Ax)
	basitrichs I	11-18 (13.38 ± 2.32) × 2-3 (2.38)	13	2/6	4.33	Te (A), Ac (T, Ax)
	basitrichs II	12-32 (22.11 ± 3.92) × 2-4 (2.38)	70	6/6	23.33	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	13-23 (18.08 ± 2.61) × 3-6 (4.2)	79	6/6	26.33	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	44-64 (52.56 ± 8.96) × 5-8 (6.3)	30	3/6	10	Te (A), Ac (A)
	microbasic p-mastigophore III	9-16 (11.55 ± 1.82) × 4-6 (4.65)	29	4/6	9.66	Te (T), Ac (Mf)
3	spirocyst	14-18 (16 ± 2.82) × 3-4 (3.5)	2	1/3	0.66	Te (T), Ac (T)
	microbasic b-mastigophore	14-23 (18.5 ± 2.38) × 3-5 (4)	26	2/3	8.66	Te (Ax)
	basitrichs II	12-30 (21.29 ± 4.2) × 2-4 (2.52)	57	3/3	19	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	12-32 (20.23 ± 4.05) × 3-6 (4.09)	182	3/3	60.66	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	35-69 (51.08 ± 9.77) × 5-7 (6.12)	25	3/3	8.33	Te (A), Ac (A)
	microbasic p-mastigophore III	10-17 (12.75 ± 2.86) × 5-6 (5.37)	8	2/3	2.66	Te (T), Ac (Mf)
Mar del Plata: La Estafeta (Buenos Aires)						
1	basitrichs II	15-27 (20.66 ± 3.6) × 2-4 (3)	12	4/4	4	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	12-25 (18.39 ± 2.7) × 3-6 (4.14)	250	4/4	83.33	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	26-33 (28.68 ± 1.96) × 3-5 (4.33)	38	4/4	12.66	Te (A), Ac (A)
2	basitrichs II	15-24 (19.5 ± 6.36) × 2-3 (2.33)	2	2/3	0.66	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	12-25 (18.34 ± 2.45) × 3-6 (4.25)	260	3/3	86.66	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	26-33 (28.86 ± 1.81) × 4-6 (4.55)	38	3/3	12.66	Te (A), Ac (A)
3	basitrichs II	17-24 (21.4 ± 2.88) × 2-3 (2.6)	5	1/3	1.66	Te (A), Ac (T, Ax)
	microbasic p-mastigophore I	15-25 (19.21 ± 2.29) × 3-6 (4.2)	226	3/3	75.36	Te (A, Ax, Mf, C), Ac (T)
	microbasic p-mastigophore II	26-34 (29.04 ± 2.09) × 3-5 (4.31)	68	3/3	22.66	Te (A), Ac (A)
	microbasic p-mastigophore III	62 × 5	1	1/3	0.33	Te (A), Ac (Mf)

S, specimen number; n, number of capsules measured; NC, proportion of cerates studied that contained cnidae. Species: Ac, *Anthothoe chilensis*; Te, *Tricnidactis errans*. Sea anemone structures: A, acontia; Ax, actinopharynx; C, column; Mf, mesenterial filament; T, tentacles.

fed on pedal discs and columns of sea anemones, because the behavioral response of the anemones prevented easy access to the tentacles. Conklin and Mariscal (1977) reported that *Spurilla neapolitana* (delle Chiaje, 1844) mainly fed on the oral disc and tentacles of the anemones *Anthopleura krebsi* Duchassaing and Michelotti, 1860 and *Anemonia sargassensis* Hargitt, 1908. In addition, Harris and Howe (1979) reported that the species *Aeolidia papillosa* (Linnaeus, 1761) had a clear preference for the tentacles of *Anthopleura elegantissima* (Brandt, 1835).

During attacks on the sea anemones *P. imperfecta* and *A. aachates*, we observed different defense responses, including tentacle and column retraction and detachment from the substrate. Similar escape responses were reported for *Anthopleura nigrescens* (Verill, 1928) from the aeolid *Herviella* sp. (Rosin 1969) and for *Actinia equina* (Linnaeus, 1758), *Anemonia sulcata* (Pennant, 1777), *A. elegantissima* (Brandt, 1835) from *A. papillosa* (Edmunds et al. 1976, Harris and Howe 1979).

In Chubut (Larralde and Punta Gales) and Mar del Plata (La Estafeta and Punta Cantera), we found similar cnidocyst types in the aeolidacean cerata, although there were differences in terms of presence/absence and abundances of some types even between individuals from the same collection site. Cnidocysts found in specimens from Chubut implied greater feeding on external rather than internal structures, which is consistent with experimental observations. On the other hand, in specimens from Mar del Plata, we observed a mix of cnidocysts from both internal and external structures of the anemones *A. chilensis* and *T. errans*. These species are small acontarian sea anemones (with respective diameters of 8-19 and 2.5-7.5 mm), and both are distributed in the low intertidal, reproduce asexually, and form packed aggregations (Pires 1988, Excoffon et al. 1997). *Spurilla* sp. is often observed on these aggregations (Garese pers. observ.); these characteristics of the prey may be the reasons for the presence of a mix of cnidocysts (internal and external) from both species.

The absence of holotrichs in the nudibranch cerata suggests that the principal prey in Mar del Plata is *A. chilensis* and not *T. errans*, which has this type of cnidocyst according to Pires (1988). Also, in accord with descriptions of cnidoms in the literature (Acuña and Zamponi 1997, Acuña et al. 2007a b), we did not take into account the species *Bunodosoma zamponii* Braga Gomes, Schama

and Solé-Cava, 2011, *Oulactis muscosa* (Drayton in Dana, 1846), or *Aulactinia marplatensis* (Zamponi, 1977) as possible nudibranch prey, because all of them have holotrichs. Column of both *O. muscosa* and *A. marplatensis* have adhesive verrucae; hence, these species are usually covered with exogenous material, like sand or shell bits (Zamponi et al. 2004), hindering predation by *Spurilla* sp. Moreover, *B. zamponii* occupies grooves and hollows in the most exposed places of the upper intertidal (Zamponi et al. 1998), whereas *Spurilla* sp. occurs in the low intertidal.

We did not observe nudibranchs directly feeding on sea anemone tentacles on any occasion; however, analysis of the cerata revealed the presence of cnidocysts from tentacles. The present study showed that various types of cnidocysts from sea anemone prey were incorporated into the cnidosacs of *Spurilla* sp. (Tables 1, 2). In other aeolidacean species that prey on hydrozoans, cnidocyst incorporation was postulated to be selective. Kepner (1943) found that *Cratena* (as *Trinchesia*) *pilata* (Gould, 1870) took up nematocysts from *Pennaria disticha* (as *Tiarella*) Goldfuss, 1820; it used only 1 type, the microbasic mastigophores, and digested and destroyed the other types. The preferred type could be considered the most effective deterrent to predators. In contrast, in work on *Cratena peregrina* (Gmelin, 1791) and its prey *Eudendrium racemosum* (Cavolini, 1785), Martin (2003) found no evidence for a selection process or digestion of a certain nematocyst type. A later study by Frick (2005) found that nematocyst uptake in aeolid nudibranchs changed according to their diet, and that their natural kleptocnidae are diverse and may vary in different individuals.

The function of kleptocnidae in aeolid nudibranchs has repeatedly been discussed; in general, they are considered defensive against predation. However, other work suggested that cnidosacs fulfill a function of storage and excretion of indigestible cnidocysts; it was also proposed that they can be used to attack cnidarian prey during the feeding process (Glaser 1910, Edmunds 1966 2009, Miller and Byrne 2000, Frick 2003 2005, Greenwood 2009, Penney et al. 2010).

Nudibranchs acquire numerous prey compounds and use them for their own defense from predators; some nudibranchs also synthesize their own chemical defenses (Cimino and Gavagnin 2006, Cimino and Ghiselin 2009). Previous chemical studies from Patagonian nudibranchs demonstrated both strategies (Munian

1997, Fontana et al. 1998, Gavagnin et al. 1999). Ciavatta et al. (1996) reported prenylphenols from the skin of the aeolid *C. peregrina*, which are completely absent from its prey, the hydroid *E. racemosum*. Chemical studies on *Spurilla* sp. from Larralde (Chubut) revealed the presence of a diol nitrile alkaloid called bursatellin in the nudibranch but not in the prey *P. imperfecta*, also indicating that the compound was not food-derived (García-Matucheski et al. 2009). The relative roles and importance of kleptocnidae versus chemical compounds obtained from cnidarians prey by aeolid nudibranchs are largely unknown and remain to be determined. In *Spurilla* sp., further studies are needed to determine whether the chemicals or the cnidocysts sequestered from its prey are effective deterrents to predators.

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REFERENCES

- Acuña FH, AC Excoffon, SR McKinstry, D Martínez. 2007a. Characterization of *Aulactinia* (Actiniaria: Actiniidae) species from Mar del Plata (Argentina) using morphological and molecular data. *Hydrobiologia* **592**: 249-256.
- Acuña FH, AC Excoffon, L Ricci. 2007b. Composition, biometry and statistical relationships between the cnidom and body size in the sea anemone *Oulactis muscosa* (Cnidaria: Actiniaria). *J. Mar. Biol. Assoc. UK* **87**: 415-419.
- Acuña FH, MO Zamponi. 1997. The use of cnidocysts for ecological races identification from sea anemones populations (Anthozoa, Actiniidae). *Iheringia Sér. Zool.* **82**: 9-18.
- Aguado F, A Marín. 2007. Warning coloration associated with nematocyst-based defenses in aeolidoidean nudibranchs. *J. Mollusc. Stud.* **73**: 23-28.
- Ciavatta M, E Trivellone, G Villani, G Cimino. 1996. Prenylphenols from the skin of the aeolid mollusc *Cratena peregrina*. *Gazz. Chim. Ital.* **126**: 707-710.
- Cimino G, M Gavagnin, eds. 2006. Molluscs: from chemoeological study to biotechnological application. Progress in molecular and subcellular biology. Heidelberg, Germany: Springer.
- Cimino G, MT Ghiselin. 2009. Chemical defense and the evolution of opisthobranch gastropods. *Proc. Calif. Acad. Sci.* **60**: 175-422.
- Conklin EJ, RN Mariscal. 1977. Feeding behavior, ceras structure, and nematocyst storage in the aeolid nudibranch *Spurilla neapolitana*. *Bull. Mar. Sci.* **27**: 658-667.
- Day RM, LG Harris. 1978. Selection and turnover of coelenterate nematocysts in some aeolid nudibranchs. *Veliger* **21**: 104-109.
- Edmunds M. 1966. Protective mechanisms in the Eolidacea (Mollusca: Nudibranchia). *Zool. J. Linn. Soc.* **47**: 27-71.
- Edmunds M. 2009. Do nematocysts sequestered by aeolid nudibranchs deter predators? - a background to the debate. *J. Mollusc. Stud.* **75**: 203-205.
- Edmunds M, GW Potts, RC Swinfen, VL Waters. 1976. Defensive behaviour of sea anemones in response to predation by the opisthobranch mollusc *Aeolidia papillosa* (L.). *J. Mar. Biol. Assoc. UK* **56**: 65-83.
- England KW. 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia). *Hydrobiologia* **216/217**: 691-697.
- Excoffon AC, MJC Belem, MO Zamponi, E Schlenz. 1997. The validity of *Anthothoe chilensis* (Lesson, 1830) (Actiniaria: Sagartiidae) and its distribution in the Southern Hemisphere. *Iheringia Sér. Zool.* **82**: 107-118.
- Fautin DJ. 1984. More Antarctic and Subantarctic sea anemones (Coelenterata: Corallimorpharia and Actiniria). *Antarct. Res. Ser.* **41**: 1-42.
- Fautin DJ. 2009. Structural diversity, systematics, and evolution of cnidae. *Toxicon* **54**: 1054-1064.
- Fontana A, C Muniain, G Cimino. 1998. First chemical study of Patagonian nudibranchs: a new seco 11-12 spongiane, tyrinnal, from the defensive organs of *Tyrinna nobilis*. *J. Nat. Prod.* **61**: 1027-1029.
- Frick K. 2003. Response in nematocyst uptake by the nudibranch *Flabellina verrucosa* to the presence of various predators in the southern Gulf of Maine. *Biol. Bull.* **205**: 367-376.
- Frick K. 2005. Nematocyst complements of nudibranchs in the genus *Flabellina* in the Gulf of Maine and the effect of diet manipulations on the cnidome of *Flabellina verrucosa*. *Mar. Biol.* **147**: 1313-1321.
- García-Matucheski S, M Carbone, C Muniain, ML Ciavatta, G Villani, M Gavagnin, G Cimino. 2009. Chemical study of aeolid nudibranchs of the genus *Spurilla* from different geographical areas. 6th European Conference on Marine Natural Products. Porto, Portugal., 78 pp.
- Garese A, FH Acuña, AC Excoffon. 2009. Cleptocnidae de *Berghia rissodominguezi* (Mollusca: Opisthobranchia, Aeolidiidae) y *Spurilla* sp. (Mollusca: Opisthobranchia, Aeolidiidae) de Mar del Plata, Argentina. XIII Congreso Latinoamericano de Ciencias del Mar-COLACMAR. La Habana, Cuba. 270 pp.
- Gavagnin M, N Ungur, F Casteluccio, C Muniain, G Cimino. 1999. New minor diterpenoid diacylglycerols from the skin of the nudibranch *Anisodoris fontaini*. *J. Nat. Prod.* **62**: 269-274.
- Glaser OC. 1910. The nematocysts of eolids. *J. Exp. Zool.* **9**: 117-142.
- Greenwood PG. 1988. Nudibranch nematocyst. In DA Hessinger, HM Lenhoff, eds. The biology of nematocysts. San Diego, CA: Academic Press, pp. 445-462.
- Greenwood PG. 2009. Acquisition and use of nematocysts by cnidarian predators. *Toxicon* **54**: 1065-1070.
- Greenwood PG, RN Mariscal. 1984a. The utilization of cnidarian nematocysts by aeolid nudibranchs: nematocyst maintenance and release in *Spurilla*. *Tissue Cell* **16**: 719-730.
- Greenwood PG, RN Mariscal. 1984b. Immature nematocyst incorporation by aeolid nudibranchia *Spurilla neapolitana*. *Mar. Biol.* **80**: 35-38.

- Grosvenor GH. 1903. On the nematocysts of aeolids. Proc. R. Soc. Lond. **72**: 462-486.
- Harris LG. 1971. Nudibranch associations as symbioses. In TC Cheng, ed. Aspects of the biology of symbiosis. Baltimore, MD: Univ. Park Press, pp. 77-90.
- Harris LG. 1973. Nudibranch associations. In TC Cheng, ed. Current topics in comparative pathobiology V, II. New York: Academic Press, pp. 213-315.
- Harris LG, NR Howe. 1979. An analysis of the defensive mechanism observed in the anemone *Anthopleura elegantissima* in response to its nudibranch predator *Aeolidia papillosa*. Biol. Bull. **157**: 138-152.
- Kepner WA. 1943. The manipulation of the nematocysts of *Pennaria tiarella* by *Aeolis pilata*. J. Morphol. **73**: 297-311.
- Lauretta D, V Häussermann, P Penchaszadeh. 2009. Re-description of *Parabunodactis imperfecta* Zamponi & Acuña, 1992 from the Patagonian Argentinean coast (Anthozoa, Actiniaria). Spixiana **2**: 167-172.
- Martin R. 2003. Management of nematocysts in the alimentary tract and in cnidosacs of the aeolid nudibranch gastropod *Cratena peregrina*. Mar. Biol. **143**: 533-541.
- Miller JA, M Byrne. 2000. Ceratal autotomy and regeneration in the aeolid nudibranch *Phidiana crassicornis* and the role of predators. Invertebr. Biol. **119**: 167-176.
- Muniain C. 1997. Moluscos Opisthobranchios de Argentina: Revisión Taxonómica y relación de Ecología Química en algunas especies patagónicas. Doctoral thesis, Univ. de Oviedo, Oviedo, España, pp. 163.
- Muniain C. 2004. Babosas marinas. In EE Boschi, MB Cousseau, eds. La vida entre mareas: vegetales y animales de las costas de Mar del Plata, Argentina. Mar del Plata, Argentina: INIDEP, pp. 271-276.
- Naville A. 1926. Notes sur les eolidis. Un eolidien d'eau saumâtre. Origine des nématocystes. Zooxanthelles et homochromie. Rev. Suisse. Zool. **33**: 251-286.
- Östman C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. Sci. Mar. **64**: 31-46.
- Penney BK, LH LaPlante, JR Friedman, MO Torres. 2010. A non invasive method to remove kleptocnidae for testing their role in defence. J. Mollusc. Stud. **76**: 296-298.
- Pires DO. 1988. *Tricnidactis errans* n. gen., n. sp. (Cnidaria, Actiniaria, Haliplanellidae), from Guanabara Bay, Rio de Janeiro, Brazil. Rev. Bras. Biol. **48**: 507-516.
- R Development Core Team. 2008. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Riemann-Zürneck K. 1975. Actiniaria des Südwestatlantik. II. Sagartiidae und Metridiidae. Helgoländer wiss. Meeresunters **27**: 70-95.
- Rosin R. 1969. Escape response of the sea anemone *Anthopleura nigrescens* (Verrill, 1928) to its predatory aeolid nudibranch, *Herviella baba* sp. nov. Veliger **12**: 74-77.
- Su Y, LJ Huang, YW Chang, HK Mok. 2009. Temporal changes in nudibranch composition at a coastal site off Penghu (the Pescadores) in the Taiwan Strait. Zool. Stud. **48**: 448-459.
- Zamponi MO, FH Acuña. 1992. Algunos hexacorallia (Cnidaria) del intermareal de Puerto Madryn y la enmienda del género *Parabunodactis* Carlgren, 1928. Neotropica **39**: 41-45.
- Zamponi MO, MJC Belém, E Schlenz, FH Acuña. 1998. Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. Physis **55**: 31-45.
- Zamponi MO, AC Excoffon, FH Acuña. 2004. Anémonas de mar y otros pólipos. In EE Boschi, MB Cousseau, eds. La vida entre mareas: vegetales y animales de las costas de Mar del Plata, Argentina. Mar del Plata, Argentina: INIDEP, pp. 93-104.