

## Larval Spirurida (Nematoda) Parasitizing Two Crab Species (*Uca uruguayensis* and *Chasmagnathus granulatus*) from the Southwest Atlantic Coast of Argentina

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**ABSTRACT:** As a result of the parasitological examination of the crabs, *Uca uruguayensis* (Ocypodidae) and *Chasmagnathus granulatus* (Varunidae), third-stage larval nematodes belonging to the family Acuariidae and to the genus *Ascarophis* were found in the crabs' hemocoel, both free and embedded in host tissues. Larval nematodes are described and illustrated here. The presence of these larval nematodes in both crab species may be influenced by the similarity in the crabs' habitat and feeding behavior. Both species of burrowing crabs inhabit the upper intertidal zones and feed on sediments, and consequently, they have similar chances of coming into contact with eggs and infective larval nematodes deposited in the feces of birds and fish definitive hosts. Since the life cycles of the larval nematodes from *U. uruguayensis* and *C. granulatus* remain unknown and because the adult stage is undescribed, they cannot be assigned to a given genus or species. The validity of a previous record of *Skrjabinoclava* sp. in *U. uruguayensis* from Bahía Samborombón is also discussed.

**KEY WORDS:** Nematoda, Acuariidae, Cystidicolidae larvae (L3), *Uca uruguayensis*, *Chasmagnathus granulatus*, southwest Atlantic coast, Argentina.

Nematodes belonging to the order Spirurida Chitwood, 1933 include a large number of genera that use crustaceans as intermediate hosts (Anderson, 2000). The identification of these larvae is difficult because there is little information regarding the development and life cycles of the great majority of the species.

Robaldo and Monserrat (1999) reported third-stage larvae of a nematode identified as *Skrjabinoclava* sp. in the crabs *Chasmagnathus granulatus* Dana (Varunidae) from Lagoa dos Patos estuary, Brazil, and *Uca uruguayensis* (Nobili) (Ocypodidae) from Bahía Samborombón, Argentina. These authors identified nematodes from *U. uruguayensis* based on examination of only 3 specimens collected during the study of the population dynamics of the parasitic isopod *Leidyia distorta* by Roccatagliata and Torres Jordá (2002).

For the present study, specimens of the fiddler crab *U. uruguayensis* from the same sample of Roccatagliata and Torres Jordá (2002) and specimens of the burrowing crab *C. granulatus* from Mar Chiquita coastal lagoon, Argentina, were examined, and 2 different morphological types of third-stage spirurid larvae were recognized.

The aim of this work is to describe, illustrate, and compare the larval nematodes found in these

crustacean hosts, using light and electron microscopy. The validity of a previous record of *Skrjabinoclava* sp. in *U. uruguayensis* and *C. granulatus* and the role of these crabs as intermediate hosts for nematodes are also discussed.

### MATERIALS AND METHODS

A sample of 12,033 *Uca uruguayensis*, collected in Bahía Samborombón (36°19'S; 56°47'W) from February 1995 to March 1996, by Roccatagliata and Torres Jordá (2002), and 218 specimens of *Chasmagnathus granulatus* collected in Mar Chiquita coastal lagoon (37°46'S; 57°27'W) from June 1992 to June 1994, were examined. The crabs were fixed in 5% formalin and stored in 70% ethanol or transported to the laboratory and examined immediately after death.

Larval nematodes were cleared in glycerin-alcohol for examination under the light microscope. Drawings were made with the aid of a camera lucida. Measurements are presented in micrometers as the mean followed by the range in parentheses, except when otherwise indicated. Due to the fact that larval nematodes collected from both crab hosts showed similar morphological features, measurements of larvae from the two host species were merged. Ten specimens of each larvae type were dried by the critical point method and examined with a scanning electron microscope (Jeol/SET100). Prevalence and mean intensity were calculated for each parasite species according to Bush et al. (1997).

In addition, voucher specimens (CHMLP 4854) of third-stage larvae of *Ascarophis marina* described by Martorelli et al. (2000) from crustaceans (*Artemesia longinaris* Batte, 1888) in Argentine waters were reexamined and compared with present specimens.

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Voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata (CHMLP), La Plata, Argentina.

## RESULTS

Two different Spirurida species were recognized, each parasitizing both crab species, *U. uruguayensis* and *C. granulatus*. Most crabs harbored only one nematode.

### Family Acuariidae

#### Railliet, Henry, and Sisoff 1912

#### Acuariinae gen. sp. third-stage larva (Figs. 1–2, 5–9)

#### Description

Based on 30 specimens from *U. uruguayensis* and 3 specimens from *C. granulatus*, the following characteristics were noted: Body length 4.35 (3.74–4.62) mm, width at midbody 120 (80–156); cuticle with slightly transverse striations along entire body; pseudolabia conical; 4 large cephalic papillae and 2 amphids located at bases of pseudolabia; vestibule elongated and cylindrical, 126 (110–138) long; nerve ring 155 (140–179) and excretory pore 275 (202–315) from anterior end; cordons long—not recurrent, originating at bases of pseudolabia and extending posteriorly, 355 (300–446) long, formed by 2 rows of spine-like plates; lateral alae extending from cervical region to last third of body; deirids simple, included in lateral alae and located at level of the muscular-glandular esophagus junction, 457 (387–518) from anterior end; muscular esophagus 294 (230–395) long and glandular esophagus 1.75 (1.54–1.98) mm long; right and left postdeirids located at 1.32 (1.15–1.55) and 1.26 (1.12–1.40) mm from posterior end, respectively; tail 156 (136–169) long, ending in a terminal appendage 13 (10–17) long, surrounded by 4 prominent protuberances, 2 dorsally and 2 ventrally directed; phasmids present at the base of tail-tip; female genital primordium near the end of the body, attached to body wall; male genital primordium elongated, 293 (140–576) long, located near esophageal-intestinal junction, 2.39 (1.55–3.93) mm from anterior end; rectum surrounded by 3 oval glandular cells (1 dorsal and 2 ventral).

#### Taxonomy summary

*Hosts:* *Uca uruguayensis* (Crustacea: Ocypodidae) and *Chasmagnathus granulatus* (Crustacea: Varunidae).

*Localities:* Bahía Samborombón (36°19'S;

56°47'W) and Mar Chiquita coastal lagoon (37°46'S; 57°27'W), Argentina.

*Prevalence and mean intensity:* 0.23% and 1.21% in *U. uruguayensis*, and 1.4 % and 1%, in *C. granulatus*.

*Site of infection in both host crabs:* hemocoel (free or embedded in host tissues).

*Specimens deposited:* 10 specimens collected from *U. uruguayensis* were deposited in the Helminthological Collection of the Museo de La Plata (CHMLP), coll. no. 5552.

#### Remarks

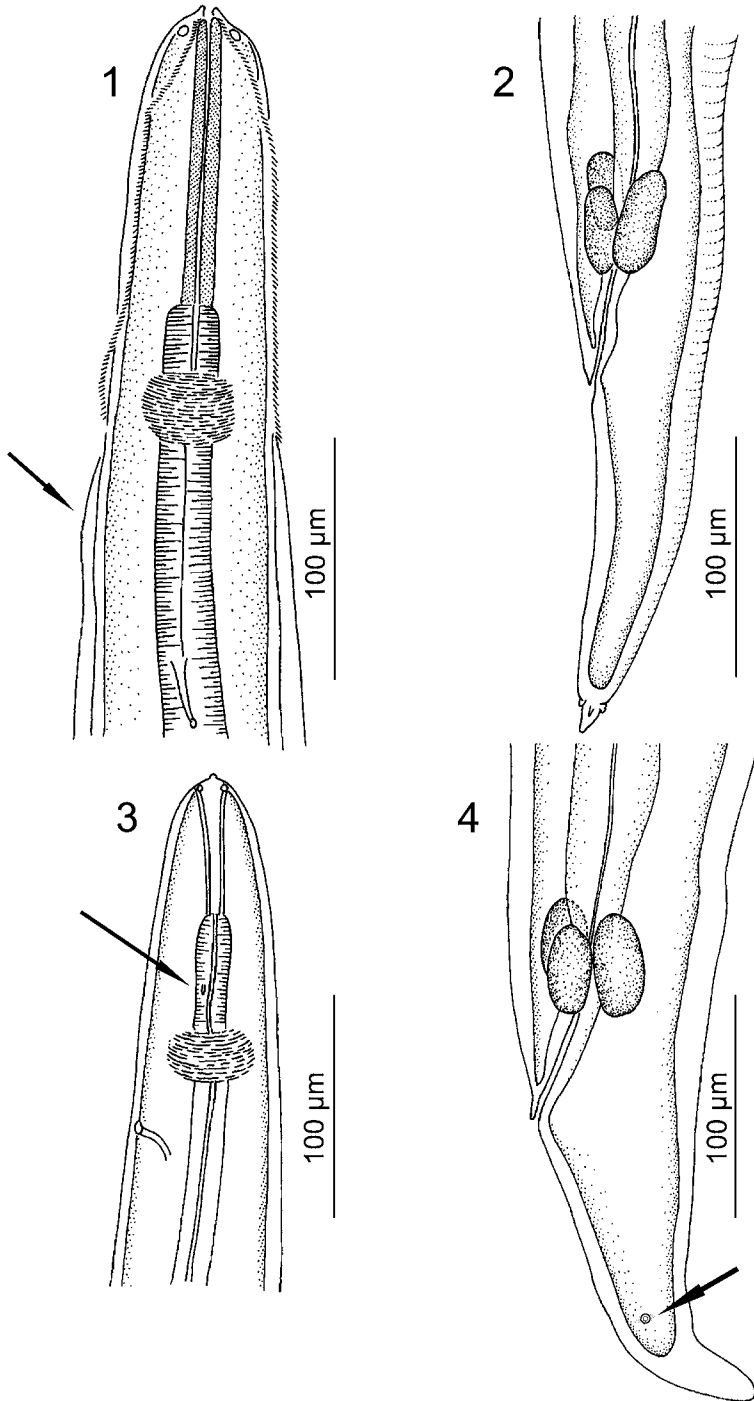
The presence of long cuticular cordons places these larval nematodes in the family Acuariidae (Chabaud, 1974). Among about 13 genera of the family Acuariidae reported parasitizing birds in South America (Vicente et al., 1995; Digiani, 1999; Etchegoin et al., 2000; Diaz et al., 2001, 2004, 2005), 4 are parasites of terrestrial birds and use insects as intermediate hosts (e.g., Orthoptera and Coleoptera) and 3 are from birds occurring in freshwater habitats. Among 6 genera parasitizing fish-eating birds, 3 genera have very short cordons, restricted to the cephalic extremity (*Paracuaria* Krishna Rao, 1951, *Skrjabinochlava* Sobolev, 1943, and *Deliria* Vicente, Pinto, and Noronha, 1980), and the other 3 genera possess longer cordons (*Skrjabinocerca* Shikhobalova, 1930, *Pectinospirura* Wehr, 1933, and *Cosmocephalus* Molin, 1858). The specimens collected in the present study have long cordons and are thus probably members of 1 of the 3 latter genera. However, because the life cycle of these nematodes from *U. uruguayensis* and *C. granulatus* remains unknown and the adult stage undescribed, they cannot be assigned to a given Acuariidae genus.

### Family Cystidicolidae Skrjabin, 1946

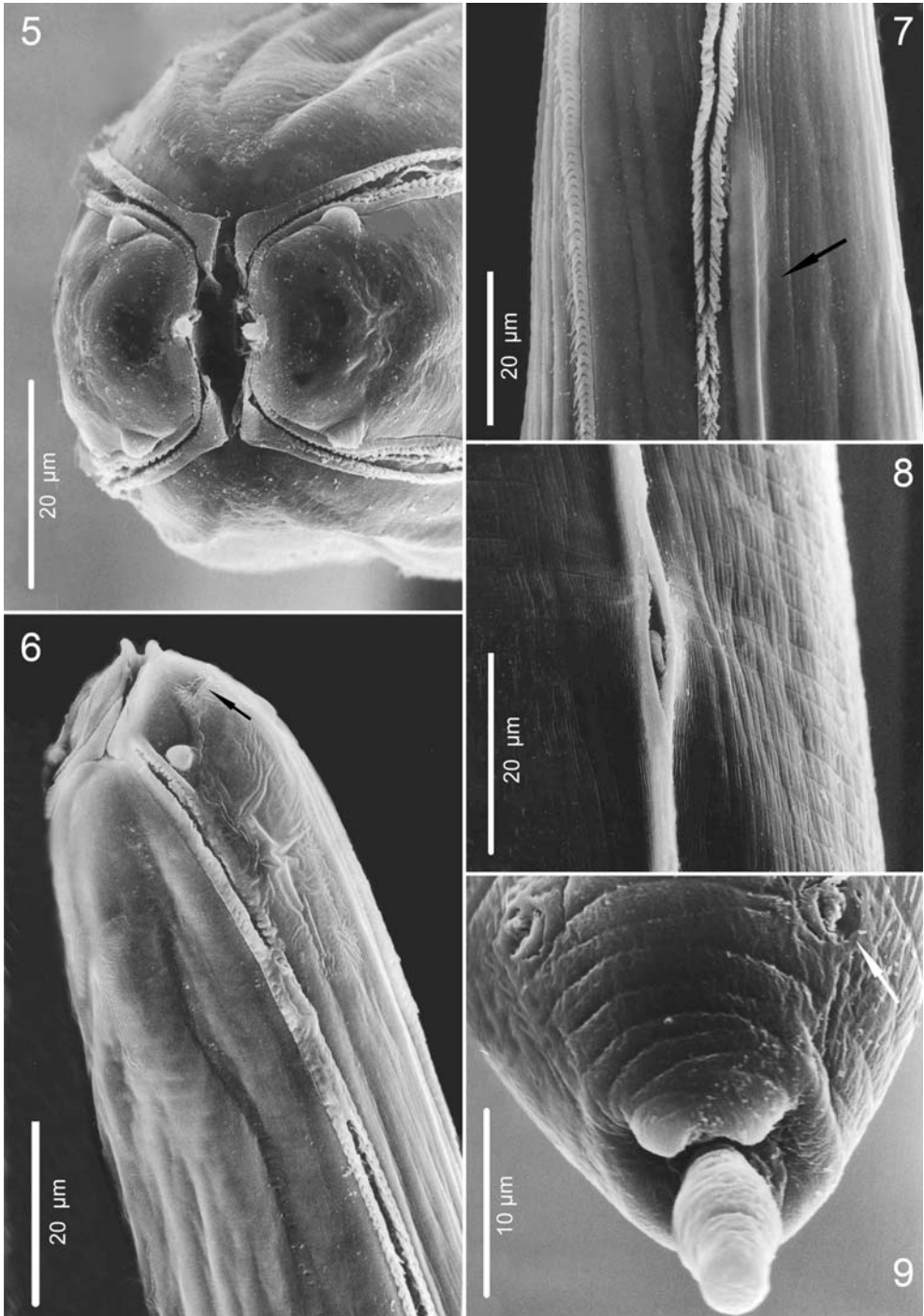
#### *Ascarophis* sp. third-stage larva (Figs. 3–4, 10–13)

#### Description

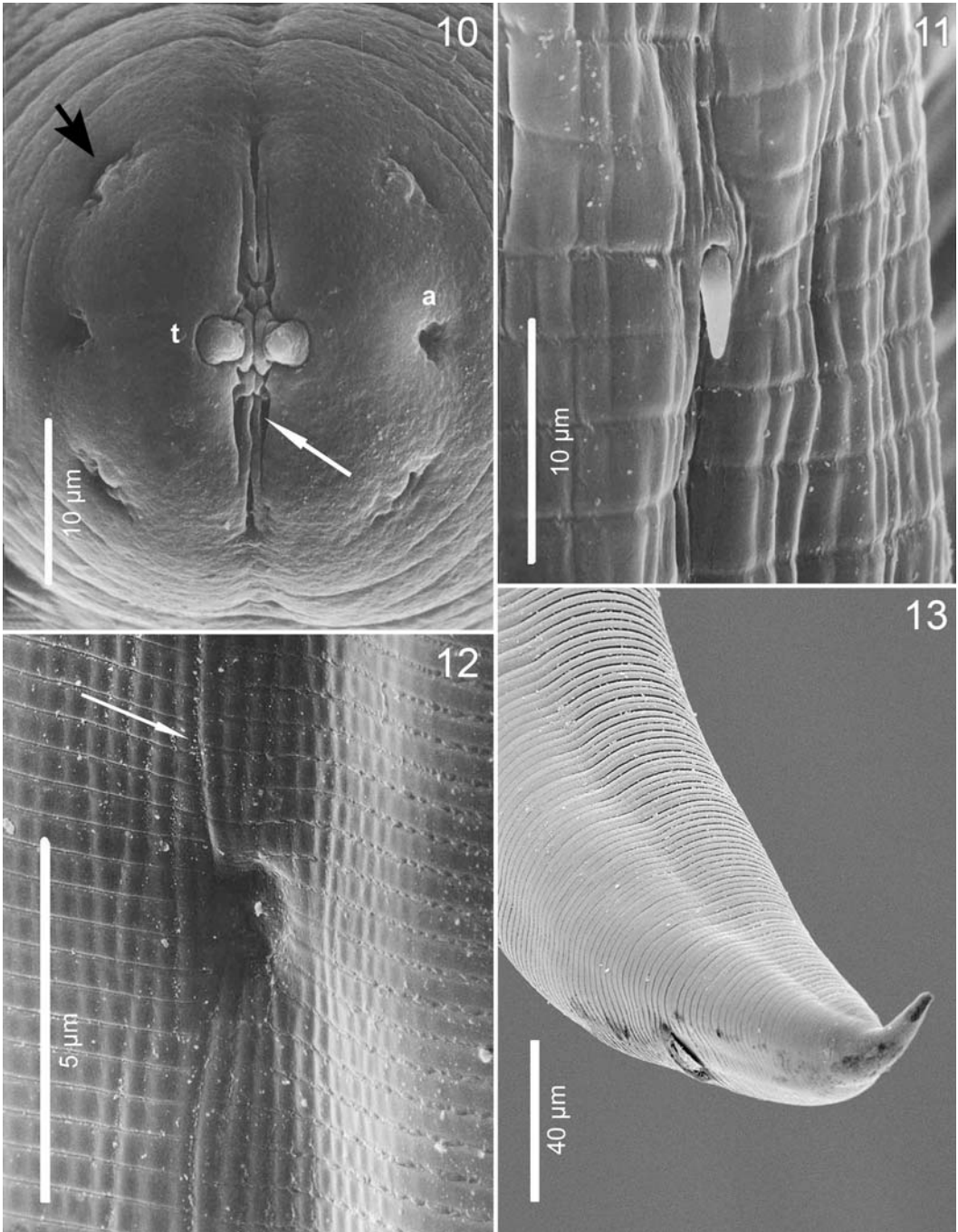
Based on 20 specimens from *U. uruguayensis* and 5 specimens from *C. granulatus*, the following characteristics were noted: Body length 7.41 (5.56–9.17) mm, width at midbody 138 (101–165) cuticle with transverse striations along the body; oral opening elongated along dorsoventral axis surrounded by 4 slightly developed lips and 2 conspicuous pseudolabia with conical terminal tooth;



**Figures 1–4.** Third-stage larval Spirurida from crabs. **1–2.** *Acuariinae* gen. sp., third-stage juvenile. **1.** Anterior end ventral view, showing cordons, excretory pore, and lateral alae (arrow). **2.** Posterior end lateral view. **3–4.** *Ascarophis* sp., third-stage juvenile. **3.** Anterior end ventral view, showing deirid (arrow) and excretory pore. **4.** Posterior end lateral view, showing glandular cells and phasmid (arrow).



**Figures 5–9.** *Acuariinae* gen. sp., third-stage larva. **5.** Anterior end apical view showing pseudolabia, cordons, and cephalic papillae. **6.** Anterior end lateral-dorsal view, showing amphids (arrow). **7.** Detail of cordons at level of beginning of lateral alae (arrow). **8.** Detail of deirid. **9.** Caudal end, showing terminal appendages and phasmid (arrow).



**Figures 10–13.** *Ascarophis* sp., third-stage larva. **10.** Anterior end apical view, showing pseudolabia, teeth (t), submedian labium (white arrow), amphids (a), and cephalic papillae (black arrow). **11.** Detail of deirid. **12.** Detail of postdeirid and lateral alae (arrow). **13.** Caudal end lateral view.

internally, each submedian labium bears elongated sublabium; 4 cephalic papillae located ventrolaterally and dorsolaterally, and 2 amphids; vestibule short and cylindrical, slightly expanded at anterior end, 51.5 (40–63) long; deirids simple and conspicuous located anterior to nerve ring, 88 (82–98) from anterior end; nerve ring 113 (90–123) and excretory pore 144 (118–157) from anterior end; esophagus divided; muscular portion shorter than glandular portion; muscular esophagus = 654 (404–910) long and glandular esophagus 1.64 (1.2–2.10) mm long; lateral alae present as a groove reaching near body end, including inconspicuous deirids and postdeirids; genital primordium = 688 (392–1,020) long, 3.60 (2.54–4.48) mm from anterior end; rectum surrounded by 3 oval cells (1 dorsal and 2 ventral); tail = 102.5 (68–123) long, ending in a conical caudal appendix 19 (12–22) long, dorsally curved; phasmids present at the base of tail-tip.

### Taxonomy Summary

*Hosts:* *Uca uruguayensis* (Crustacea: Ocypodidae) and *Chasmagnathus granulatus* (Crustacea: Varunidae).

*Localities:* Bahía Samborombón (36°19'S; 56°47'W) and Mar Chiquita coastal lagoon (37°46'S; 57°27'W), Argentina.

*Prevalence and mean intensity:* 0.17% and 1% in *U. uruguayensis*, and 3.2% and 1% in *C. granulatus*.

*Site of infection in both host crabs:* hemocoel (free or embedded in host tissues).

*Specimens deposited:* 6 specimens collected from *U. uruguayensis* were deposited in the Helminthological Collection of the Museo de La Plata (CHMLP), coll. no. 5553.

### Remarks

The general morphology and the presence of an oral opening elongated along the dorsoventral axis surrounded by 4 slightly developed lips, 2 conspicuous pseudolabia with conical terminal tooth, and 4 submedian labium bearing elongated sublabium, place these larval nematodes in the family Cystidicolidae, genus *Ascarophis*.

Only 3 species of *Ascarophis* have been reported in the southwestern Atlantic, and only *Ascarophis marina* Szidat 1961 has been reported from our study area (Ivanov et al., 1997). Martorelli et al. (2000) collected third-stage larvae of *A. marina* from the crustaceans *Artemesia longinaris* (Argentinean stiletto

shrimp) and *Peisos petronkevitchi* (shrimp) in Argentinean waters. Our specimens from crabs can be distinguished from *A. marina* by their shorter and wider body (5.56–9.17 vs. 7.6–19 mm and 101–165 vs. 40–90, respectively), as well as a longer muscular esophagus (404–910 mm vs. 210–500 mm). In addition, the specimens from crabs have a conical tail dorsally curved, a feature not observed in *A. marina* (Martorelli et al., 2000). Since the life cycle of the *Ascarophis* larvae from *U. uruguayensis* and *C. granulatus* remains unknown and the adult stage undescribed, they cannot be assigned to a given species.

### DISCUSSION

Acuariid nematodes are mainly parasites of fish-eating birds and use crustaceans as intermediate hosts in aquatic life cycles (Anderson, 2000). In experimental infections, fiddler crabs (*Uca* spp.) were found to be intermediate hosts for *Skrjabinoclava inornatae* Wong and Anderson, 1988 (Wong et al., 1989). Third-stage larvae of *Ancyracanthopsis winegardi* Wong and Anderson, 1990 were found encapsulated in the hemocoel of naturally infected fiddler crabs (Wong and Anderson, 1990).

Robaldo and Monserrat (1999) identified the larval nematodes from *C. granulatus* (collected from Lagoa dos Patos, Brazil) and *U. uruguayensis* (collected from Bahía Samborombón, Argentina) as *Skrjabinoclava* sp. However, in their specimens, the deirids were located posterior to the nerve ring, the muscular and glandular esophagus were similar in length, the tip-tail was conical and dorsally bowed, and they did not mention the presence of grooves or lateral alae. The morphological and morphometric characteristics of these specimens clearly disagree with the description of *Skrjabinoclava* larvae given by Wong et al. (1989). The specimens described by Robaldo and Monserrat (1999) actually have morphological features similar to the larvae described as *Ascarophis* sp. in the present study.

Knowledge of the transmission and development of the Cystidicolidae has increased considerably. Adults are parasites of marine and freshwater fishes, and several aquatic insects or crustaceans (e.g., Amphipoda, Decapoda) serve as intermediate hosts. Larvae can be free in the hemocoel or invade the tissues and became encapsulated (Anderson, 2000). Species of *Ascarophis* van Beneden, 1981 parasitize the digestive tract of marine and estuarine fishes (Ko, 1986). Third-stage larvae found in marine decapods

have been assigned to *Ascarophis* by several authors (e.g., Poinar and Kuris, 1975; Moravec et al., 2003).

*Ascarophis nototheniae* Johnston and Mawson, 1945, *Ascarophis brasiliensis* Magahales Pinto, Vicente, and Noronha, 1984, and *A. marina* Szidat, 1961 have been reported from the southwestern Atlantic (Ivanov et al., 1997). Martorelli et al. (2000) proposed the life cycle of *A. marina* in Argentinean waters. These authors collected adult *A. nototheniae* from the fishes *Parona signata* (Carangidae) and *Urophycis brasiliensis* (Gadidae), and third-stage larvae from the shrimp *Artemesia longinaris* and *Peisos petronkevitchi*. The finding of the third-stage larvae of *Ascarophis* sp. in *C. granulatus* and *U. uruguayensis* enlarges the range of hosts and the geographical distribution of this genus.

The presence of same species of larval nematodes (i.e., Acuariidae gen. sp. and *Ascarophis* sp.) in *U. uruguayensis* and *C. granulatus* may be influenced by the similarity in the crabs' habitat and feeding behavior. In fact, *U. uruguayensis* is distributed along the Atlantic coast from Rio de Janeiro, Brazil, to Quequén Salado, Argentina (34°–38°S); this range overlaps the geographic range of *C. granulatus* (28°–42°S) (Daleo et al., 2003). Both species of burrowing crabs inhabit the upper intertidal zones (Spivak et al., 1994; Iribarne and Martínez, 1999) and feed on sediments (Botto and Iribarne, 2000), and, consequently, they have similar chances of encountering eggs and infective larval nematodes deposited on the muddy and sandy beaches and on the bottom of the lagoons.

In Bahía Samborombón, Iribarne and Martínez (1999) reported 4 species of shorebirds feeding on *U. uruguayensis*, and Favero et al. (2001) indicated *C. granulatus* as a food item for the birds *Larus atlanticus*, *Larus maculipennis*, and *Haematopus palliatus* in Mar Chiquita coastal lagoon (Buenos Aires province, Argentina). As previously stated, acuariid nematodes of aquatic birds use crustaceans as intermediate hosts in their life cycles, and, thus, both species of crabs examined in the present study can be considered as potential hosts for the nematodes parasitizing shorebirds feeding in the upper intertidal zones. Regarding *Ascarophis* sp., at present, only *C. granulatus* has been cited as a food item for the sciaenid fish *Micropogonias furnieri* (Cousseau et al., 2001). In this case, crabs could be infected when the water invades their habitat during high tides. Moravec et al. (2003) reported a similar composition (i.e., an acuariid and a species of *Ascarophis*) in the crab *Macrophthalmus hirtipes* (Ocyrodidae) from New Zealand.

The observed prevalence for both nematodes in

the present study agrees with the large range of prevalence (2% to 90%) reported by different authors for Spirurida larvae (Gestal et al., 1999; Moravec et al., 2003). Fluctuations in the prevalence of third-stage larvae in intermediate hosts may be due to seasonality, host size, host sex, and geographical distribution (Poinar and Kuris, 1975). However, these factors have not yet been evaluated for the larval nematodes of *U. uruguayensis* and *C. granulatus*.

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