

PARASITES AND EPIBIONTS OF GRAPSID CRABS IN BAHÍA BLANCA ESTUARY, ARGENTINA

BY

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ABSTRACT

Epibionts and parasites were recovered from the grapsid crabs *Cyrtograpsus angulatus* and *Neohelice granulata* in the Bahía Blanca estuary, Argentina. Among epibionts, we identified a filamentous bacterium, the protozoan *Epistylis* sp., and the copepod *Neocancrincola platensis* on the gills; and the barnacles *Balanus amphitrite* and *Balanus glandula* as well as the bryozoan *Conopeum reticulum* on the caparace. Among endoparasites, we identified the acanthocephalan *Profilicollis chasmagnathi* in the hindgut, nematodes of the subfamily Acuariinae and *Ascarophis* sp. in the haemocoel, and an encysted cyclophyllidean cestode in the gut wall. Among microphallid digeneans, we found four metacercariae: *Maritrema bonaerensis* in the gills, muscle, haemocoel, and gonads, *Maritrema orensensis* in the gills, *Levinseniella cruzi* in the gonads, and *Odhmeria* sp. in the muscle. Except for *P. chasmagnathi*, *N. platensis*, and *M. bonaerensis*, all other epibionts and digeneans represent new host records. This paper also presents novel information regarding the possible sites of infection for *M. bonaerensis*. Our results add relevant data about possible life cycles of helminths in the study area.

RESUMEN

Se recuperaron epibiontes y parásitos de los cangrejos grápsidos *Cyrtograpsus angulatus* y *Neohelice granulata* en el estuario de Bahía Blanca, Argentina. Entre los epibiontes, se identificaron una bacteria filamentosa, el protozoo *Epistylis* sp. y el copépodo *Neocancrincola platensis* en las branquias, y los cirripedios *Balanus amphitrite* y *Balanus glandula* y el briozoo *Conopeum reticulum* en el caparazón. Entre los endoparásitos, se identificaron el acantocéfalo *Profilicollis chasmagnathi* en el intestino posterior, los nematodos Acuariinae y *Ascarophis* sp. en el hemocele y un cestodo cyclophyllideo en la pared intestinal. Entre los digeneos microphallidos, se encontraron cuatro metacercarias: *Maritrema bonaerensis* en las branquias, el músculo, el hemocele y las gónadas, *Maritrema orensensis* en las branquias, *Levinseniella cruzi* en las gónadas y *Odhmeria* sp. en el músculo. Excepto por *P. chasmagnathi*, *N. platensis* y *M. bonaerensis*, todos los otros epibiontes y digeneos representan nuevos registros para los hospedadores. Además, este trabajo presenta nuevos

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sitios de infección para *M. bonaerensis* y revela nuevos datos sobre los posibles ciclos de vida de los helmintos en el sitio de estudio.

INTRODUCTION

Temperate southwestern Atlantic salt marshes are inhabited by dense populations of two grapsid crabs, *Cyrtograpsus angulatus* Dana, 1851 and *Neohelice granulata* (Dana, 1851) (cf. Olivier et al., 1972; Spivak et al., 1994). Although research has been conducted on parasites and epibionts infecting both crabs (Mañé-Garzón & Sobota, 1974; Holcman-Spector et al., 1977; Martorelli, 1986, 1989; Martorelli & Schuldt, 1990; Etchegoin & Martorelli, 1997; Silva et al., 2003, 2007; Cremonte et al., 2007; La Sala et al., 2009a), there is still an important gap in our knowledge about the parasitological fauna of these crabs in the Bahía Blanca estuary in the southwest of the Buenos Aires province.

Therefore, the objectives of this study were (1) to identify and report prevalence of parasites and epibionts on *C. angulatus* and *N. granulata* from the Bahía Blanca estuary, and (2) to compare our results with those from studies conducted at other sites.

MATERIAL AND METHODS

The Bahía Blanca estuary is the second largest in Argentina, after the Río de La Plata estuary. It is characterized by extensive tidal flats, a complex system of tidal channels, patches of low salt marshes, and shallow islands. Two small tributaries flow into the estuary (Perillo & Piccolo, 1991).

Crabs were collected at Puerto Cuatros (38°44'S 62°22'W) in the innermost part of the estuary. Samples were collected in March, April, July, September, and November in 2008, and in February and April 2009. *Cyrtograpsus angulatus* (n = 65) and *Neohelice granulata* (n = 36) were collected using hand nets and crab traps. The specimens were transported to the laboratory alive and killed by freezing (−20°C) for 20 min. Sex and carapace width were recorded. Crabs were first examined externally for epibionts. Gills, foregut, hepatopancreas, and gonads were removed and examined under a stereomicroscope for the presence of parasites. The muscle of the thorax and chelipeds was examined. Symbionts and parasites were fixed in 10% formalin, stored in 70% ethanol, and studied under a compound light microscope. Some digeneans were excysted. Measurements here reported are based on the study of 10 specimens, except for *Levinseniella cruzi* Travassos, 1920 (6 specimens recovered). Measurements are presented in μm as minimum and maximum range followed by their mean between parentheses.

Voucher specimens of *Odhneria* sp. (6197) and *Maritrema orensensis* Cremonte & Martorelli, 1998 (6198) were deposited in the Museo de La Plata Helminth Collection, Argentina.

RESULTS

A total of 65 individuals of *Cyrtograpsus angulatus* was collected (61% female). Specimens were 14-40 mm (27.4 ± 5.8) in width. Of these, 40% had one or more species of epibionts on the carapace, gills, or gill chambers, and 98% had one or more species of parasites in the gills, gut wall, hindgut, haemocoel, muscle, and gonads. A total of 36 specimens of *Neohelice granulata* was collected (58% female). Specimens were 25-37 mm (30.0 ± 3.2) in width. Of these, 31% had at least one epibiont, and endoparasites were found in all of the crabs examined.

In the following, we report ectosymbionts and parasites found in *C. angulatus* and *N. granulata* and provide morphological descriptions for some of those species.

ECTOSYMBIONTS

Kingdom BACTERIA

Filamentous Bacteria (fig. 1A)

Description. — Non-branching filaments attached to the cuticle of the gills. Cells near the base of the filaments 1.45-2.14 (1.76) long and 5.42-6.64 (6.03) wide, those near the middle 1.54-3.14 (2.00) long and 7.70-9.18 (8.27) wide, and those near the apex 2.09-3.57 (2.82) long and 7.88-8.57 (8.26) wide. Terminal gonidia 3.36-4.54 (4.05) long and 5.39-9.50 (7.28) wide.

Hosts. — *N. granulata* and *C. angulatus*.

Site of occurrence in host crabs. — Gills.

Prevalence. — 11% (*N. granulata*) and 12% (*C. angulatus*).

Remarks. — Filamentous bacteria are frequently found on the gills of crabs (Stentiford & Feist, 2005). Heavy infestations may clog the gills and interfere with respiration. Based on cell morphology, it is possible that these bacteria belong to the genus *Leucothrix*. These filamentous bacteria use body surfaces, gills, and eggs of their host crabs as substrates and they have also been found on the gills and eggs of the blue crab, *Callinectes sapidus* Rathbun, 1896 (cf. Shields & Overstreet, 2007).

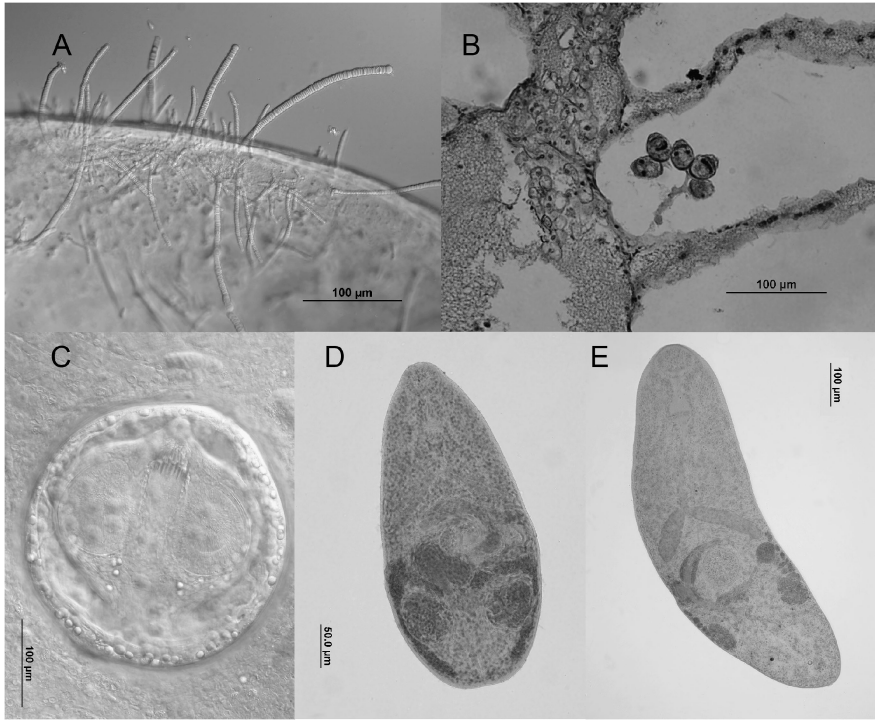


Fig. 1. A, filamentous bacteria attached to the cuticle of the gills of *Neohelice granulata* (Dana, 1851); B, *Epistylis* sp. on gills of *N. granulata*; C, cyclophyllidean cestode in gut wall of *Cyrtograpsus angulatus* Dana, 1851; D, metacercaria of *Maritrema orensensis* Cremonte & Martorelli, 1998 from *N. granulata*; E, metacercaria of *Odhneria* sp. from *C. angulatus*.

Kingdom PROTOZOA
 Phylum CILIOPHORA
 Class OLIGOHYMENOPHOREA
 Order PERITRICHIDA
 Family EPISTYLIDAE
Epistylis sp. (fig. 1B)

Description. — Colonial. Short and dichotomously branched stalk; inverted bell-shaped zooids, elongated and ovoid, 52-77 (60) by 34-50 (43) in vivo, with a conspicuous lip. Single, contractile vacuole located below the peristomal disc, horseshoe-shaped macronucleus located in upper third of the body. Micronucleus not observed.

Hosts. — *N. granulata* and *C. angulatus*.

Site of occurrence in host crabs. — Gills.

Prevalence. — 8% (*N. granulata*) and 35% (*C. angulatus*).

Remarks. — The presence of one peristomal ciliary row and non-contractile stalks ending in a single zooid places this protozoan in the genus *Epistylis* (cf. Colin et al., 1983).

Kingdom ANIMALIA
 Phylum BRYOZOA
 Class GYMNOLEAMATA
 Order CHEILOSTOMATA
 Family MEMBRANIPORIDAE
Conopeum reticulum (Linnaeus, 1767)

Hosts. — *N. granulata* and *C. angulatus*.

Site of occurrence in host crabs. — Caparace.

Prevalence. — 3% (*N. granulata*) and 2% (*C. angulatus*).

Remarks. — The presence of colonial zooids with granular margins and small, triangular zooids at the distal end of normal zooids agrees with the morphological characteristics of *C. reticulum*. This is a cosmopolitan species in fouling communities (Orenzans et al., 2002) and it has been previously reported in benthic communities of the Bahía Blanca estuary (Bremec, 1989).

Phylum ARTHROPODA
 Subphylum CRUSTACEA
 Class MAXILLOPODA
 Infraclass CIRRIPIEDIA
 Family BALANIDAE
Balanus amphitrite Darwin, 1854
Balanus glandula Darwin, 1854

Hosts. — *N. granulata* and *C. angulatus*.

Site of occurrence in host crabs. — Caparace.

Prevalence. — 20% (*N. granulata*) and 8% (*C. angulatus*).

Remarks. — The presence of distinct, vertical bands of purple stripes on the capitulum plates agrees with *B. amphitrite*, while white or grayish walls agree well with *B. glandula*. Both barnacles are exotic in coastal and estuarine habitats of Argentina (Orensanz et al., 2002).

Subclass COPEPODA
 Infraclass NEOCOPEPODA
 Order HARPACTICOIDA
 Family CANCRINCOLIDAE

Neocantrincola platensis Mañé-Garzón & Sobota, 1974

Hosts. — *N. granulata* and *C. angulatus*.

Site of occurrence in host crabs. — Gill chambers.

Prevalence. — 21% (*N. granulata*) and 8% (*C. angulatus*).

Remarks. — The presence of 2 caudal ramus setae, the absence of inner setae on the second segment of the exopodite of the first leg, and a long inner seta on the distal segment of the fifth leg agree with characteristics of *N. platensis*. This species was described by Mañé-Garzón & Sobota (1974) in gill chambers of *N. granulata* on the Uruguayan coast.

PARASITES

Phylum NEMATODA

Class SECERNENTEA

Order SPIRURIDA

Family CYSTIDICOLIDAE

Ascarophis sp.

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Body cavity.

Prevalence. — 4% (*N. granulata*) and 8% (*C. angulatus*).

Remarks. — The general morphology and the presence of 4 slightly developed lips, 2 pseudolabia with conical terminal teeth, and 4 submedian labia bearing elongated sublabia, places this larval nematode in the family Cystidicolidae, genus *Ascarophis*. Previous work by Cremonte et al. (2007) reported *Ascarophis* sp. larvae in *N. granulata* from Samborombón Bay (Argentina, Buenos Aires).

Family ACUARIIDAE

Subfamily ACUARIINAE

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Body cavity.

Prevalence. — 3% (*N. granulata*) and 6% (*C. angulatus*).

Remarks. — The presence of four cuticular cordons with plates, a striated cuticle, conical pseudolabia with 4 cephalic papillae, and 2 amphids places this larval nematode in the family Acuariidae (cf. Chabaud, 1974), and most likely in the subfamily Acuariinae (cf. Anderson, 2000). Acuariid nematodes have been reported for both species of crabs (Cremonte et al., 2007; La Sala et al., 2009a).

Phylum ACANTHOCEPHALA
Class PALAEACANTHOCEPHALA
Order POLYMORPHIDA
Family POLYMORPHIDAE

Proflicollis chasmagnathi (Holcman-Spector, Mañé-Garzón & Del-Cas, 1977)

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Hindgut.

Prevalence. — 47% (*N. granulata*) and 20% (*C. angulatus*).

Remarks. — The characteristics of these cystacanths, i.e., proboscis armed with 18-20 longitudinal rows of 8-9 hooks each, four tubular cement glands, and the body measurements, agree with those of *P. chasmagnathi*. This species was described by Holcman-Spector et al. (1977) in *N. granulata* from the Santa Lucía estuary in Uruguay. *Proflicollis chasmagnathi* was also reported in *C. angulatus* from Mar Chiquita coastal lagoon (Martorelli, 1989), and in *C. angulatus* and *N. granulata* from the Bahía Blanca estuary (La Sala & Martorelli, 2007).

Phylum PLATYHELMINTHES
Class CESTODA
Subclass EUCESTODA
Order CYCLOPHYLLIDEA (fig. 1C)

Description. — Cysts oval, 290-339 (318) by 262-309 (288), surrounded by an outermost wall (host-induced) 2.60-12.27 (5.66) thick, and an innermost one (cysticeroid-induced) 1.74-4.91 (2.78) thick. Cysticeroids bearing an apical sucker, 4 lateral suckers 97-128 (109) by 67-92 (79), and a rostellum with prominent hooks invaginated into a rostellar sac. The largest calcareous corpuscles measured 8.68-12.84 (9.83) in diameter.

Host. — *C. angulatus*.

Site of infection. — Gut wall.

Prevalence. — 8%.

Remarks. — According to Cheng (1978), a scolex with 4 muscular suckers and a rostellum place this cysticeroid in the order Cyclophyllidea. Although family-level identification was not possible, the rostellar hooks closely resemble those of *Alcataenia* sp. recovered from the intestine of Olrog's gull, *Larus atlanticus* Olrog, 1958, from the Bahía Blanca estuary (La Sala, 2010).

Class TREMATODA
Subclass DIGENEA
Order PLAGIORCHIIDA
Family MICROPHALLIDAE

TABLE I

Prevalence of *Maritrema bonaerensis* Etchegoin & Martorelli, 1997 in grapsid crabs of the Bahía Blanca estuary

	Prevalence (%)	
	<i>Cyrtograpsus angulatus</i> Dana, 1851 (n = 65)	<i>Neohelice granulata</i> (Dana, 1851) (n = 36)
Gill	83	97
Haemocoel	83	94
Muscle	23	31
Gonad	8	8

Maritrema bonaerensis Etchegoin & Martorelli, 1997

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Gills, haemocoel (free or embedded into host tissues), muscle, and ovary (table I).

Prevalence. — 100% (*N. granulata*) and 94% (*C. angulatus*).

Remarks. — The body measurements and vitelline follicles forming a complete ring encircling the uterus and testes agree with metacercariae of *M. bonaerensis*. All measurements are in accordance with those reported by Etchegoin & Martorelli (1997), except for a shorter prepharynx (7-60 vs. 30-60) and a longer ovary (53-97 vs. 50-60) in *M. bonaerensis* from our study. Metacercariae developed eggs in saline solution at 37°C. We observed two types of cysts of *M. bonaerensis*; the first one was found in the gills, it was round, small, 277-343 (308) by 230-320 (271) and it has two concentric layers; the second one was found in the haemocoel, it was round but bigger than the first, 340-457 (381) by 300-433 (352), and it has only one layer. The latter agrees with cysts of *M. bonaerensis* described by Etchegoin & Martorelli (1997).

Maritrema cf. orensensis Cremonte & Martorelli, 1998 (fig. 1D)

Description. — Metacercarial cyst oval and small, 270-307 (290) by 180-247 (217), with two hyaline layers, the innermost 3-7 (5) and the outermost of 3-10 (7) thick. Body covered with spines, 275-366 (310) long and 131-198 (162) wide at acetabular level. Oral sucker subterminal, 32-41 (37) by 27-44 (34). Prepharynx 12-34 (25) long. Pharynx 17-27 (23) long and 12-19 (14) wide. Oesophagus 14-34 (23) long. Intestinal caeca 64-95 (74) long and 8-13 (10) wide, not reaching the acetabular level. Ventral sucker 20-29 (24) by 22-36 (26). Testes oval, symmetrical, postovarian, 31-34 (33) long and 33-58 (47) wide. Cirrus sac curved, 83-136 (97) long and 15-29 (23) wide. Ovary 22-29 (23) long and 13-53 (32) wide. Vitellarium

U-shaped forming oval follicles between testes and ovary. Excretory vesicle Y-shaped.

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Gills.

Prevalence. — 70% (*N. granulata*) and 70% (*C. angulatus*).

Remarks. — Body morphology and vitelline follicles forming an incomplete ring encircling the uterus and testes agree with *M. orensensis* (cf. Cremonte & Martorelli, 1998). The life history of *M. orensensis* remains undescribed and experimental infestations or molecular studies are needed to confirm our identification.

Levinseniella cruzi Travassos, 1920

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Gonad.

Prevalence. — 11% (*N. granulata*) and 14% (*C. angulatus*).

Remarks. — The body measurements and the presence of a genital atrium containing 10 atrial pockets agree with metacercariae of *L. cruzi*. The metacercariae developed eggs in saline solution at 37°C. With the exception of a smaller ovary (37-54 by 56-68 vs. 52-60 by 90-100) observed in *L. cruzi* from our study, all other measurements agree with those reported by Martorelli (1988) who first reported *L. cruzi* parasitizing the gonads, muscle, and connective tissue of the fresh-water shrimp *Palaemonetes argentinus* Nobili, 1901. This shrimp is one of the most widely distributed decapods in Argentina, occurring both in fresh water and brackish water areas (Spivak, 1997). *Levinseniella cruzi* has been reported in hosts from both fresh water and brackish environments (Martorelli, 1988; Martorelli & Ivanov, 1996). Thus, this digenean appears to have a wide range of salinity tolerance and a wide array of potential hosts.

Odhneria sp. (fig. 1E)

Description. — Metacercarial cyst 330-513 (387) by 303-383 (346) with two hyaline layers, the innermost 7-13 (11) and the outermost 12-47 (22) thick. Excysted metacercariae covered with spines, 450-800 (623) long and 133-210 (178) wide at the testes level. Oral sucker subterminal, 63-100 (74) by 73-100 (83). Prepharynx 20-60 (39) long. Pharynx 33-73 (54) long and 30-47 (37) wide. Oesophagus 67-193 (127) long and 7-20 (11) wide. Intestinal caeca 80-132 (101) long and 28-48 (36) wide, extending to acetabular level. Ventral sucker 55-93 (72) by 63-107 (85). Testes oval, symmetrical, postovarian, 42-102 (72) long and 19-61 (38) wide. Cirrus sac curved, 110-169 (147) long and 10-27 (15) wide. Ovary oval, 25-53 (39) long and 19-51 (33) wide. Vitellarium formed by oval follicles

located along the lateral margins of the body. Excretory vesicle V-shaped with lateral evaginations on the wall, opening into the posterior body extremity. Flame cell formula $2[(2 + 2) + (2 + 2)] = 16$.

Hosts. — *N. granulata* and *C. angulatus*.

Site of infection in host crabs. — Haemocoel and muscle.

Prevalence. — 53% (*N. granulata*) and 59% (*C. angulatus*).

Remarks. — According to Schell (1970), the presence of a uterus posterior to and between the testes and vitelline follicles in the lateral bands anterior to the testes place this metacercariae in the genus *Odhneria*. Stunkard (1979) studied the life cycle of *Odhneria odhneri* and found that the metacercariae encyst in the prawn *Palaemonetes vulgaris*. Experimental infections by Stunkard (1979) revealed post-infection presence of metacercariae of 17 by 14 in size. In natural infections, he observed cysts of different sizes, fully developed except for the maturation of reproductive organs.

DISCUSSION

Here, we report the presence of one bacterium, two barnacles, one bryozoan, one protozoan, one copepod, two nematodes, one acanthocephalan, one cestode, and four digeneans in *Cyrtograpsus angulatus* and *Neohelice ganulata*. This is the first report of filamentous bacteria, barnacles, bryozoans, and protozoans for these grapsid crabs in the Bahía Blanca estuary. Silva et al. (2003, 2007) reported bacteria, filamentous fungi, and peritrichid protozoans on eggs of *C. angulatus* and *N. granulata* from Mar Chiquita, an estuarine environment some 260 km northeast of Bahía Blanca estuary. The copepod *Neocancrincola platensis* was reported before in *N. granulata* (cf. Mañé-Garzón & Sobota, 1974). Regarding our finding of larval cestodes in *C. angulatus*, this is the first report of cysticercoids in this crab.

Previous research conducted in Mar Chiquita reported the presence of *Microphallus szidati* Martorelli, 1986 (Digenea, Microphallidae) in *C. angulatus* (cf. Martorelli, 1986, 1989; Martorelli & Schuldt, 1990). However, so far this species was not found in crabs from the Bahía Blanca estuary.

Regarding *Maritrema bonaerensis*, this microphallid was previously found in the gills and haemocoel of both crab species in the Mar Chiquita lagoon and in the Bahía Blanca estuary (Etchegoin & Martorelli, 1997; La Sala et al., 2009a). However, this is the first work to report *M. bonaerensis* in muscle and gonads. We found two different types of *M. bonaerensis* cysts in tissues of the studied crabs. Those encysted in the gills were smaller than those in other tissues, which might be explained by the migration of the metacercariae from the entrance site in the

crab's gills to other tissues and compartments, as suggested by Heard & Overstreet (1983) and Saville & Irwin (2005).

This is the first report of *Maritrema* cf. *orensensis* parasitizing gills, *Levinseniella cruzi* parasitizing gonads, and *Odhneria* sp. parasitizing muscle and haemocoel of *N. granulata* and *C. angulatus*. Previous studies conducted in the studied area reported intestinal infections by adults of *M. bonaerensis*, *M. orensensis*, *O. odhneri*, and *L. cruzi* in Olrog's gull (*Larus atlanticus*), adults and chicks from the Bahía Blanca estuary (La Sala et al., 2009b). It is worth noting that Olrog's gull adults are feeding specialists during their breeding season in this area, preying mainly on *N. granulata* and *C. angulatus*, which they feed to their young (Delhey et al., 2001), thus exposing themselves and their chicks to helminth infections.

Then, the parasitological findings presented here contribute significantly to our knowledge about possible life cycles of microphallid helminths in this poorly studied environment.

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