First report of pigmentation dystrophy in terrestrial isopods, Atlantoscia floridana (van Name) (Isopoda, Oniscidea), induced by larval acanthocephalans¹

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ABSTRACT. The observation of pigmentation alteration in isopod crustaceans induced by acanthocephalans, known as pigmentation dystrophy, has been documented in North America in species of the aquatic genera *Asellus* Geoffroy, 1764, *Lirceus* Rafinesque-Schmaltz, 1820, and *Caecidotea* Packard, 1871, and in Europe, in *Asellus*. Recently, three depigmented specimens of *Atlantoscia floridana* (van Name, 1940), a terrestrial isopod, occurring from Florida, USA to northern Argentina were found showing pigmentation dystrophy and harboring larval acanthocephalans. Photographic documentation of live and preserved, infected isopods is presented. Morphometric data and photomicrographs of the male, unencysted cystacanth specimen which allowed its placement in the genus *Centrorhynchus* Lühe, 1911 are presented. This is the first record of the phenomenon of pigmentation dystrophy in terrestrial isopod crustaceans, the first record of *A. floridana* infected by an acanthocephalan and the first record of a species of *Centrorhynchus* in a terrestrial isopod.

KEY WORDS. Crustacea, cystacanth, intermediate hosts, pigmentation alteration, southern Brazil.

Acanthocephalans as adults are intestinal parasites of vertebrates, and their life cycle includes arthropods as intermediate hosts where their larvae, acanthellae and cystacanths, develop. Occasionally vertebrates participate in the life cycle as paratenic hosts harboring cystacanths. The paratenic hosts will serve as a trophic bridge between the intermediate and the definitive hosts carrying the cystacanths to the definitive host.

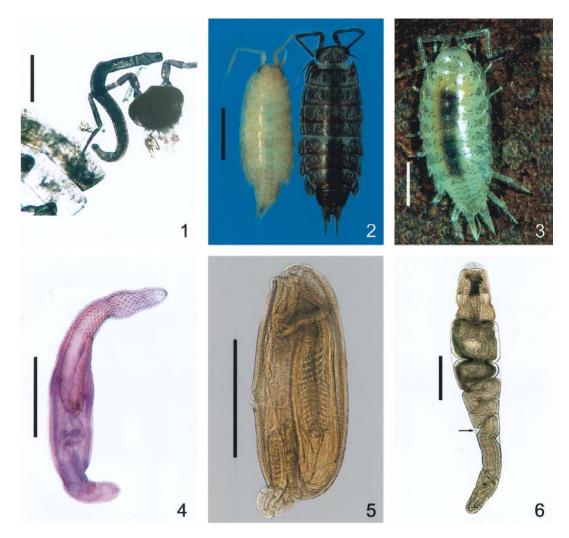
Pigmentation alteration of isopod and amphipod crustaceans induced by larval acanthocephalans, known as "color dichromatism", "depigmentation", "non-pigmentation", or more properly, "pigmentation dystrophy", as suggested by OETINGER & NICKOL (1981), has been documented for North America in species of the aquatic isopod genera *Asellus* Geoffroy, 1764, by SEIDENBERG (1973), CAMP & HUIZINGA (1979) and OETINGER & NICKOL (1981, 1982); *Lirceus* Rafinesque-Schmaltz, 1820, by OETINGER & NICKOL (1981, 1982), and *Caecidotea* Packard, 1871, by OETINGER (1987). DEZFULI *et al.* (1994), documented this alteration in Europe, also in species of *Asellus*.

In terrestrial isopods, MOORE (1984) has pointed out that *Armadillidium vulgare* (Latreille, 1804) infected with cystacanths of *Plagiorhynchus cylindraceus* (Goeze, 1782) did not show pig-

mentation dystrophy, but displayed behavior alterations. SIDDIKOV (1987) reported a species of *Hemilepistus* Budde-Lund, 1885 and a species of *Porcellio* Latreille, 1804 as being infected by cystacanths of *Centrorhynchus lanceoides* (Petrochenko, 1949), which GOLVAN (1956) had transferred to his newly erected genus *Sphaerirostris* Golvan, 1956, but did not mention any alteration concerning pigmentation and behavior. So, terrestrial isopods, up to the present, had not been found displaying pigmentation dystrophy induced by larval acanthocephalans.

Atlantoscia floridana (van Name, 1940), a terrestrial isopod of the family Philosciidae, has a wide, known geographical distribution (Florida, USA to northern Argentina) (LEISTIKOW & WÄGELE 1999). This species of woodlouse is found in practically all Brazilian coastal states, and, according to LEMOS DE CASTRO (1985), is the predominant isopod in the habitats where it occurs.

The present paper registers for the first time the phenomenon of pigmentation dystrophy induced by larval acanthocephalans in terrestrial isopods, and *A. floridana* harboring a larval acanthocephalan of the genus *Centrorhynchus* Lühe, 1911.



Figures 1-6. Atlantoscia floridana, infected and uninfected and cystacanths of *Centrorhynchus* sp.: (1) live, unencysted cystacanth of *Centrorhynchus* sp., head with antennae and two unpigmented pereionites of *A. floridana*, bar = 1 mm; (2) fixed, infected specimen (left) showing pigmentation dystrophy and uninfected specimen (right) of *A. floridana*, bar = 2 mm; (3) live, infected specimen of *A. floridana*, showing pigmentation dystrophy, bar = 1 mm; (4) unencysted cystacanth of *Centrorhynchus* sp., from *A. floridana*, stained in Delafield's hematoxylin, bar = 1 mm; (5) encysted cystacanth of *Centrorhynchus* sp., cleared in Amann's lactophenol; (6) live, unstained, encysted cystacanth of *Centrorhynchus* sp., showing envelope of parasite origin (arrow), bars = 500 μ m.

MATERIAL AND METHODS

More than 8000 specimens of *A. floridana*, collected between 1990 and 2002, were examined for pigmentation dystrophy, in southern Brazil, including many localities in the states of Santa Catarina and Rio Grande do Sul (map in ARAUJO *et al.* 1996). The three depigmented isopods were collected, two of them on the Campus of the Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil, (30°03'S, 51°07'W), in November 2001 and September 2002, respectively, and the other depigmented specimen was collected in the coast town of Rondinha, Rio Grande do Sul. The larvae were fixed in AFA (Ethanol, Formalin, Glacial Acetic Acid) and stained in Delafield's hematoxylin (HUMASON 1979). Four hundred and twenty six pigmented specimens of *A. floridana* were also examined after clarification in Amann's lactophenol. Measurements are in micrometers (μ m), unless otherwise indicated; averages follow ranges within parentheses; widths were taken at widest point. All measurements for proboscis hooks were made in full lateral profile. The only unencysted cystacanth was deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, State of Rio de Janeiro, Brazil (Catalog N°: 36196 male); the infected isopods were deposited in the Coleção de Crustáceos, Departamento de Zoologia, UFRGS (Catalog N°: 3551 female, 2507 male and 3552 female). The pigmented,

uninfected specimens of *A. floridana* were also deposited (Catalog N° 3553). Four adult specimens of *C. tumidulus* deposited in the CHIOC N° 1899, 1900, 1901, and 1902, were examined.

RESULTS

Three specimens of *A. floridana* were found with pigmentation alteration. The first and the third specimens (Figs 1 and 3), collected alive during litter triage, showed pigmentation dystrophy in the head, pereionites and pereiopods. It was possible to see the faint, irregular pigmented areas as well as the dark contents (food) in the digestive system. The second isopod with pigmentation alteration (Fig. 2, left) was found fixed in ethanol, among many others with the normal, dark pigmentation, deposited in the crustacean collection (Fig. 2, right).

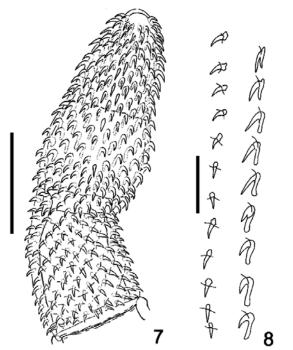
Upon dissection, all the isopods with pigmentation alteration were found to harbor one larval acanthocephalan (cystacanth) each, in the hemocoel of the pereion. The 426 isopods with normal pigmentation collected from the same location as the first and third specimens, were negative for larval acanthocephalans.

The live, unencysted cystacanth (Figs 1 and 4), was whitish and moved actively, it had the proboscis inserted in tissues of the anterior region of the hemocoel. The second fully encysted cystacanth (Fig. 5) could not be removed from the cyst because the isopod was already fixed in ethanol. The third cystacanth, alive at dissection of the host, was whitish and recently encysted having only the envelope of parasite origin (Fig. 6, arrow).

The cystacanths found clearly belong to *Centrorhynchus* by having the typical proboscis of the genus, with three portions, the middle one inflated, and a constriction at the insertion of the proboscis receptacle (Fig. 7).

The unencysted cystacanth (Figs 1 and 4) was a male specimen: trunk cylindrical, tapering anteriorly, widest at level of the proboscis receptacle, inflated, anterior portion 1.57 mm long, 530 wide; narrower, invaginable (in cystacanths and juveniles) posterior portion, called "hindtrunk" from now on (Figs 13-14), was 550 long, 294 wide. Middle third of proboscis inflated, with the largest hooks, tapering anteriad, with a constriction at receptacle insertion, 1.04 mm long [229 wide in anterior third; 330 wide in middle third (at inflation); 250 wide at receptacle insertion; 330 wide in posterior third (at base)], with 28 rows of 20-22 hooks each, 9 are rooted hooks and 13 are rootless spines. Anterior 1-2 hooks in each row 5-7.5 long, anterior manubrium present; next 4-5 hooks with simple roots. Apical hooks 30-40 (37) long; remainder 32.5-37.5 (35.6) long. Spines 22.5-27.5 (25.2) long. Roots of largest hooks 45-57.5 (53.1) long. Neck short, not measurable in this specimen. Proboscis receptacle double-walled, extending to equator of the inflated portion of the trunk, 870 long, 224 wide; proboscis ganglion ellipsoid, just bellow the line between neck and trunk. Lemnisci with approximately equal lengths, shorter than proboscis receptacle, 700 and 750 long, respectively. Testes ellipsoid, in tandem, between middle and posterior thirds of the inflated portion of the trunk; anterior testis 170 from the bottom of proboscis receptacle.

The live encysted cystacanth (Fig. 6) was photographed and measured before fixation. It was 3.13 mm long, 445 wide (maximum width). The second, fixed, encysted cystacanth was 959 long, 392 wide (maximum width).



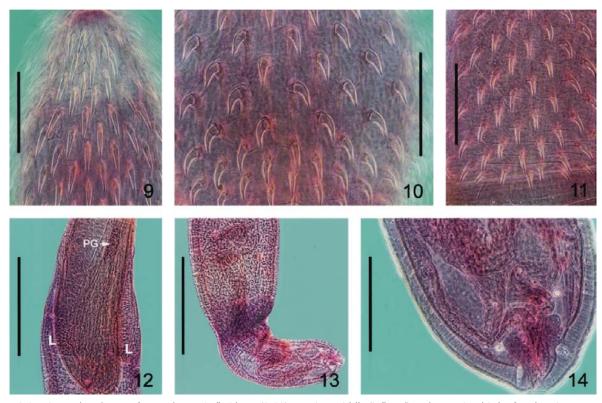
Figures 7-8. *Centrorhynchus* sp. from *Atlantoscia floridana*: (7) proboscis, bar = $500 \ \mu m$; (8) one longitudinal row with 9 hooks and 12 spines, bar = $100 \ \mu m$.

DISCUSSION

The effects of acanthocephalans on their intermediate hosts were discussed by various authors and range from increased conspicuousness, either by the larvae acquiring pigmentation or by pigmentation dystrophy of the host, which results from the interference of the acanthocephalan with the metabolic pathway for ommochrome pigmentation in the isopod hosts (OETINGER & NICKOL 1982) to castration. Recently FULLER *et al.* (2003) reported pigmentation and behavior alteration in termites due to acanthocephalans.

YAMAGUTI (1963) listed four species of Centrorhynchus as parasites of birds in Brazil. AMIN (1985) included 75 species in Centrorhynchus, most of them parasites, as adults, in birds of prey of the orders Falconiformes and Strigiformes, and, more rarely, in mammals. RICHARDSON & NICKOL (1995) have added one more species of Centrorhynchus parasite of owls for North America. DE BURON & GOLVAN (1986) have listed the known intermediate hosts of acanthocephalans and indicated only three species of terrestrial isopods of the genera Armadillidium Brandt, 1833 and Porcellio as intermediate hosts for P. cylindraceus. Centrorhynchus and Sphaerirostris were indicated, generically, as having terrestrial isopods and insects (one unconfirmed record) as intermediate hosts in their life cycles. SCHMIDT (1985) has indicated only one intermediate host for species of Centrorhynchus, and even so, it was an orthopteran of the superfamily Acridoidea, not a crustacean.

TRAVASSOS (1926), revising the Centrorhynchinae listed the following species of *Centrorhynchus* as occurring in Brazil: *C. tumidulus* (Rudolphi, 1819), *C. giganteus* Travassos, 1921, *C.*



Figures 9-14. *Centrorhynchus* sp., from *Atlantoscia floridana*: (9-11) anterior, middle (inflated) and posterior thirds of proboscis, respectively (phase contrast), bars = 125 μ m; (12) proboscis receptacle with proboscis ganglion (PG) and lemnisci (L); (13) middle portion of trunk, showing testes and hindtrunk (phase contrast); (14) distal portion of hindtrunk, showing invaginated copulatory bursa (phase contrast), bars = 500 μ m.

opimus Travassos, 1925, later transferred to *Sphaerirostris*, *C. polymorphus* Travassos, 1925 and *Centrorhynchus* sp. of JOHNSTON (1916). He indicated that these species use paratenic hosts (amphibians and reptiles) for the cystacanths, not mentioning any intermediate hosts.

RODRIGUES (1970) has recorded encysted cystacanths of an undetermined species of *Centrorhynchus*, in the common gecko, *Hemidactylus mabouia* (M. de J.) from the city of Rio de Janeiro, while DE FABIO (1982) recorded encysted cystacanths of *Centrorhynchus* sp. in several species of three genera of Leptodactylinae from the rural area of the city of Rio de Janeiro.

The CHIOC does not hold any cystacanth of *Centrorhynchus* spp. from intermediate hosts. So far, all records of cystacanths attributable to the genus *Centrorhynchus* were made in paratenic hosts, amphibians and reptiles. A survey of all deposited cystacanths in the CHIOC showed 17 lots with indication of amphibians [6 records for *Leptodactylus ocellatus* (Linnaeus, 1758) and 3 records for *Hyla* sp.] and reptiles [3 records, generically referred as snakes, 2 for *Drymobius bifossatus* (readdi, 1820)], 1 for *Herpetodryas sexcarinatus* [incorrectly identified, being either *Pseustes sexcarinatus* (Wagler, 1824) or *Chironius carinatus* (Linnaeus, 1758)], both occurring in southeastern Brazil, and 1 for *Ameiva ameiva* (Linnaeus, 1758) (today considered a complex formed

by many subspecies) as paratenic hosts. TORRES & PUGA (1996) and PUGA & TORRES (1999) recorded encysted cystacanths in paratenic hosts from Chile, which they identified as *Centrorhynchus* sp., although their drawings show that the acanthocephalans might belong to *Sphaerirostris* for having the proboscis with a spherical anterior portion and a larger number of hooks in the longitudinal rows.

Comparing the shape and oncotaxy of the proboscis of the larvae found in *A. floridana* (Figs 4, 8-11) with that reported from *H. mabouia* (paratenic host) in Rio de Janeiro by RODRIGUES (1970) is possible to see that the shape of the proboscis is markedly different, being wider in the anterior third, while that reported by DE FABIO (1982) is more similar by having the proboscis with an inflated middle third and a short hindtrunk, but still having the apical third of the proboscis wider than that being presently reported.

Comparing the proboscis of adult *C. tumidulus* in the male specimen (CHIOC N° 1899) and those in the slides CHIOC N° 1900, 1901, 1902, all collected from "anu-preto" or "ani" *Crotophaga ani* (Linnaeus, 1758) (Aves, Cucculidae), in Rio de Janeiro by Dr. Lauro Travassos, with that found in *A. floridana* (Figs 4, 7, 9-11) is possible to see differences which do not warrant the specific determination of the present specimen as *C. tumidulus*. The proboscides differ in size, although the ante-

rior third is slender and the middle third is inflated, the hooks in the apical third are stouter, having a wider base (11 μ m) and shorter blade (34 μ m) in the adult specimens (CHIOC 1899), while the spines are longer (12 μ m). The hindtrunk of the cystacanth found in the third dystrophic specimen of *A*. *floridana* (Fig. 6), collected from the same area as the first, is much longer (Fig. 4). The hindtrunk of the specimens of *C*. *tumidulus* (CHIOC N° 1899) is markedly longer (8.25 mm and 9.12 mm in the male and the female specimens, respectively). There must be a pronounced allometric growth of this region of the trunk after the cystacanth infects the final host, in this case *Crotophaga ani*.

The comparison of *C. albidus* Meyer, 1933, from an unidentified falconid bird of Paraguay, shows that although the number of longitudinal rows of hooks and the number of hooks in each longitudinal row are similar, the oncotaxy and general appearance of the proboscides are different: nine rooted anterior hooks and 12 posterior, rootless spines in our specimen and seven anterior, rooted hooks and 13 posterior, rootless spines in *C. albidus*, allows the separation of both species. In addition, the lemnisci are shorter than the proboscis receptacle in present cystacanth specimen and much longer, reaching the anterior testis, in *C. albidus*. Having only one cystacanth specimen with the everted proboscis from these three isopods makes it impossible to appreciate any intraspecific variation and to ascertain the specific identity of these larvae.

Experiments will be conducted with specimens of *A*. *floridana* raised on a regular basis in the Carcinology Laboratory to provide more cystacanths for study and to induce pigmentation dystrophy in crustaceans of different age classes, in addition to allow experimental infections of definitive hosts.

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