Natural infection by *Procyrnea uncinipenis* (Nematoda, Habronematidae), a parasite from rheas, an autoctone bird from South America, in emus *Dromaius novaehollandiae*, a ratite from New Zealand

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The present study reports a natural infection of emus, *Dromaius novaehollandiae*, by the nematode *Procyrnea uncinipenis*. Five adult emus from a scientific breeding farm at North Fluminense State University located in the city of Campos dos Goytacazes, Rio de Janeiro state, Brazil were necropsied, and their gastrointestinal tract were collected and examined for the presence of parasites from October 2013 to November 2015. Two of the five (40%) emus necropsied were infected with nematodes, and a portion of the nematodes were processed for light microscopy. In addition, two other nematodes (a male and a female) were prepared for scanning electron microscopy. In a female bird, one nematode was collected in the proventriculus and two nematodes in the gizzard and in the male bird four nematodes were collected in the gizzard. The morphological and morphometric analyzes allowed to identify the nematodes as *P. uncinipenis*, this being the first report of an infection by *P. uncinipenis* in emus. Therefore, we infer that these emus were naturally infected by nematodes that were considered specific to rheas.

There are two species of rheas in South America, the greater rhea, *Rhea americana* Linnaeus, 1758, and the lesser rhea, *Rhea pennata* (d’Orbigny 1834). Of these, *R. americana* has been of increased interest for farming since the 1990s, receiving attention in South America, North America and Europe1. This species is composed of five subspecies distributed in South America: *R. americana americana* (northeastern to southeastern Brazil), *R. americana intermedia* (southeastern Brazil and Uruguay), *R. americana nobilis* (eastern Paraguay), *R. americana araneipes* (western Paraguay, eastern Bolivia, and southwestern Brazil) and *R. americana albescens* (northeastern and eastern Argentina)2. These birds are found in open and stream areas3, and the presence of parasitic infections in these species is common, particularly those caused by the nematodes *Procyrnea uncinipenis* (Molin, 1860) and *Deletrocephalus dimidiatu*s Diesing, 1851, *Deletrocephalus cesarpintoi* Vaz, 1936 and *Paradeletrocephalus minor* (Molin, 1861)4-5, both of which are considered host-specific.

The emu, *Dromaius novaehollandiae* (Latham 1790), is widely distributed throughout the Oceania continent and is one of the most characteristic components of the modern Australian avifauna6. There are three living subspecies: *D. novaehollandiae novaehollandiae* (central and south Queensland, Victoria and Southern Australia); *D. novaehollandiae woodwardi* (Northwestern and Western Australia and northern territory), and *D.
**Material and methods**

Five adult emus (3 males and 2 females) from a scientific breeding farm regulated by the governmental agency IBAMA under number 18981-2 and approved by Ethics Committee at the Universidade Estadual do Norte Fluminense (North Fluminense State University) located in the city of Campos dos Goytacazes, State of Rio de Janeiro, Brazil were necropsied after natural death, and the gastrointestinal tract were collected and examined for the presence of parasites from October 2013 to November 2015. All applicable institutional, national and international guidelines for the care and use of animals were followed. These birds live in proximity to *R. americana* from the same breeding. The contents of the proventriculus and gizzard were passed through a sieve with a 75 μm mesh, and the mucosa was observed under a stereomicroscope (Opton TIM-2T, China). The koin layer was removed and observed for the presence of nematodes. The nematodes that were found were washed in a saline solution (0.09% NaCl). A portion of the nematodes were processed for light microscopy, and two (a male and a female) were prepared for scanning electron microscopy.

**Optical microscopy.**  The nematodes were fixed in A.F.A. (70° GL ethanol, 93%; formaldehyde, 5%; glacial acetic acid, 2%) at 70 °C, for 48 h, transferred to a solution containing 70% ethanol and 5% glycerin, cleared and mounted on slides with lactophenol (one part distillated water, two parts glycerin, one part lactic acid, one part phenic acid) and observed under a light microscope.

Measurements were performed to the nearest micron (mean ± S.D. (range)) and were conducted on three mature adult specimens and 10 embryonated eggs in utero. Measurements were conducted with an Axioplan Zeiss light microscope (Carl Zeiss, Germany) equipped with a Canon Power-Shot A640 digital camera (Canon, China) and Zeiss AxionVision Sample Images Software (Carl Zeiss, Germany) for image analysis. Specimens deposited in the Harold W. Manter Parasite Collection at the University of Nebraska-Lincoln (UNL/USA), registration number HWML 67092, were examined for comparative purposes. Representative specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (Rio de Janeiro, Brazil) under the registration number CHIOC 38941.

**Scanning electron microscopy.**  The nematodes were fixed for 2 h in 2.5% glutaraldehyde, 4% freshly prepared paraformaldehyde, and 5 mM calcium chloride in 0.1 M cacodylate buffer, pH 7.2. The nematodes were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer. The samples were dehydrated in an acetone series, critical-point dried with CO₂, sputter-coated with gold and examined under a Zeiss 962 scanning electron microscope (SEM) operating at 15 kV.

**Ethical approval.**  All applicable institutional, national and international guidelines for the care and use of animals were followed.

**Results**

Two of the five (40%) analyzed emus (one male and one female) were infected with nematodes. In one bird (female), one nematode was collected from the proventriculus and two from the gizzard, under the koin layer. In another bird (male), four nematodes were collected from under the gizzard koin layer. No gross pathology was observed.

The morphological and morphometrical analyses identified the nematodes as *P. uncinipenis*. The nematodes were large and whitish in vivo and had two lateral lips with denticles and two interlabia, four labial papillae and two amphids (Fig. 1a). The esophagus was divided into an anterior muscular portion and a posterior glandular portion. Only one female specimen was measured, which had a total body length approximately 21,724 by 734 wide. Buccal cavity 55 long by 38 wide. Muscular esophagus 357 long by 93 wide, and glandular esophagus 4874 long by 306 wide. Distances from nerve ring, excretory pore and cervical papillae to anterior end were approximately 289, 1368 and 1401, respectively. Anus and vulva with a transverse slit (Fig. 1b,c) opening at 236 and 1484 from posterior end, respectively. Female posterior end with two lateral phasmds and a circular structure at the tip tail (Fig. 1c). Eggs (n = 10) 26 ± 1.31 long, ranging from 24 to 28, by 43 ± 2.36 wide, ranging from 40 to 48.

Two males were measured, and the total body length ranged from 14,855 to 20,861 long by 624 to 625 wide, buccal cavity 47–65 long by 42–60 wide, muscular esophagus 313–516 long by 104–108 wide, glandular esophagus 3305–3442 long by 264–266 wide, nerve ring at 422–432 from anterior end. Excretory pore was not observed in the specimens. Spicules unequal in size and shape, with a proportion of approximately 1:4. Left spicule long and thin, measuring 3224 long, with a more robust spicule head (Fig. 2a). Distal tip ended sharply (Fig. 2b). Spicule head shorter (Fig. 2c). Right spicule short and thick, measuring 741 long, with a curved distal end, similar to a hook, and a dilation at the tip (Figs. 1d, 2d). Gubernaculum well chitinized, “v” shaped (Fig. 2d), 145 long.

*Procyrnea uncinipenis* is a nematode that infect the gizzard and proventriculum of rheas, *Rhea americana*, a native bird from South America. These nematodes have been reported in rheas from Brazil⁷–¹¹ and Argentina¹². Another species, *Procyrnea choique*¹³, was described from another species of rhea, *R. pennatta*, from Argentina.

The present study reports a natural infection of emus, *D. novaehollandiae*, by the nematode *P. uncinipenis*. 
Discussion

The morphology, observed by light and scanning electron microscopy, and the morphometry of the nematodes collected from *D. novaehollandiae* are similar to those of the nematode species that infect *R. americana* in South America, *P. uncinipenis*, which shows that this exotic bird can host this parasite from rheas, a native bird of the continent. This is the first report of an infection by *P. uncinipenis* in emus. Other studies that evaluated this species of parasites in emus have not reported the presence of *P. uncinipenis*\(^{14,15}\). There are reports of infection by other nematode species, including *Chandlerella quiscali*\(^{16}\), *Baylisascaris* spp.\(^{17}\), *Cyathostoma variegatum*\(^{18}\), *Dromaestrongylus bicuspis*, *Trichostrongylus tenuis*, and *Syngamus trachea*\(^{19}\).

This report of *P. uncinipenis*, a parasite from *R. americana*, in emus bred in captivity in Brazil together with rheas shows that these birds developed an adaptation to this nematode parasite, which was considered to be host-specific. In rheas, there can be a high intensity infection, with more than 400 nematodes infecting the proventriculus and gizzard, causing widespread necrosis accompanied by a hemorrhagic appearance. The nematodes deeply penetrate the gizzard glands\(^5\). Although the emus had few specimens of *P. uncinipenis* in the gizzard (\(n = 4\)), infections with a high parasite load can occur and lead to the death of the birds.

The female and male specimens of *P. uncinipenis* that infected the emus in the present study are smaller than those from *R. americana* and larger than *P. choique* from *R. pennata* (Tables 1 and 2). However, the morphology

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**Figure 1.** Scanning electron microscopy of *Procyrnea uncinipenis* (Nematoda, Habronematidae) from *Dromaius novaehollandiae* (Aves, Casuariidae). (a) Anterior end showing lateral pseudolips (pl), interlabia (i), cephalic papillae (p), amphidia (a), pseudolip denticles (arrow) and lateral lip denticles (head arrow); (b) right spicule, distal end (arrow); (c) female tip tail showing two lateral phasmids (head arrow), a circular structure (arrow) and the anus (a); (d) vulva opening (arrow). Bars: A–B: 25 µm, C: 15 µm, D: 50 µm.
observed by light and scanning electron microscopy is similar to *P. uncinipenis*, a parasite from *R. americana*. These differences in nematode size are probably due to early infection in emus. The low prevalence (2 of 5 analyzed birds) and low parasite load (one bird with 3 nematodes and another with 4 nematodes) shows that this ratite species, *D. novaehollandiae*, originally from New Zealand, become naturally infected by this nematode species from another ratite from South America, *R. americana*. Thus, farmers and zoological gardens from Oceania should pay attention to infection with *R. americana* in their flock by using effective sanitary control measures.

The intention should be to avoid the introduction of this parasite, which has the capacity to infect emus. The consequences of this parasite in other birds are unknown. In rheas, this parasitosis can lead to death.

*Procyrnea uncinipenis*, as a Spirurid, has an arthropod as intermediate host, which is unknown. So, it is not known if this parasite can complete its life cycle, if introduced in Oceania.

### Table 1. Measurements, in microns, of male specimens of *Procyrnea uncinipenis*, a parasite of rhea. *P. uncinipenis* present study (n = 2).

| Character                  | *P. uncinipenis* present study (n = 2) | *P. uncinipenis* | *P. uncinipenis* | *P. uncinipenis* | *P. waltoni* | *P. choique* |
|----------------------------|----------------------------------------|------------------|------------------|------------------|--------------|--------------|
| Body total length          | 14,855–20,861                          | 22,546–30,980    | 17,750–21,770    | 25,000–28,000    | 20,000       | 8250–9850    |
| Body width                 | 624–625                                | 659–827          | 600–740          | 600–700          | 700          | 350–400      |
| Buccal cavity deep         | 47–65                                  | 46–68            | 55–63            | 62               | 120–130      | 30–40        |
| Buccal cavity wide         | 42–60                                  | 24–40            | 67–84            | 37               | –            | 25–30        |
| Muscular esophagus length  | 313–516                                | 351–455          | 300–360          | 400–420          | 425–450      | 200–300      |
| Muscular esophagus width   | 104–108                                | 74–108           | 70–83            | –                | 40           |              |
| Glandular esophagus length | 3305–3442                              | 2685–3568        | 2660–3650        | 3220–3400        | 2600–2900    | 2640–3560    |
| Glandular esophagus width  | 264–266                                | 164–249          | 170–230          | –                | 80–120       |              |
| Nerve ring                 | 422–432                                | 382–490          | 330–390          | 420              | 360–400      | 120–200      |
| Excretory pore              | –                                      | 529–658          | 500–610          | –                | 250–290      |              |
| Cervical papillae           | –                                      | 361–411          | 290–340          | –                | 140–180      |              |
| Right spicule length       | 741 (n = 1)                            | 740–982          | 660–800          | 700–720          | 300–420      | 300–360      |
| Left spicule length        | 3224 (n = 1)                           | 3206–3774        | 3000–3700        | 3050–3170        | 2400–2650    | 970–1050     |
| Right spicule left spicule | 1:4                                    | 1:4              | 1:4              | 1:4              | 1:8          | ~ 1:4        |
| Gubernaculum length        | 145                                    | 102–169          | 110–140          | 100              | –            | 60–80        |
Table 2. Measurements, in microns, of female specimens of Procyrnea uncinipenis, a parasite of rhea.

After analyzing the nematode specimens collected from the gizzard of emus, a bird from Oceania that were introduced in Brazil for commercial and ornamental purposes, along with rheas, a native bird from South America, the present study can infer that the emus were naturally infected by nematodes that were considered specific to rheas.

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**Author contributions**

N.B.E. prepared the images and wrote the main manuscript text. S.S.M.G. measured the parasites and wrote the main manuscript text. F.C.R.O. performed the necropsy and wrote the main manuscript.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

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