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New data on eggshell structure of capillariid species: a SEM perspective

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Abstract

Capillariidae is a group of nematode parasites of vertebrates with a complex taxonomy. The structure of the eggshell, which was indicated as the most important characteristic for identification of genus or species through eggs, is very diverse among genera. The visualization and characterization of eggshell by light microscopy (LM) are a challenging task since different planes of the egg surface are needed. Nevertheless, categories of eggshell ornamentation were proposed by LM: smooth, punctuated, reticulated type I, and reticulated type II. The present study aimed to characterize the eggshell structure of Capillariidae species, parasites of mammals and avians, deposited in a helminthological collection using scanning electron microscopy (SEM). Institutional Biological Collections are taxonomic repositories of specimens described and strictly identified at the species level by systematics specialists. SEM eggshell images were obtained from 12 species belonging to 5 genera (*Aonchotheca*, *Baruscapillaria*, *Capillaria*, *Echinocoleus*, *Eucoleus*) and compared to their respective LM images. Eggshell patterns observed using SEM were associated categories of eggshell ornamentation previously proposed by LM images. The SEM data indicate that eggshell categories are not in agreement with capillariid genera or sites of infection. However, the study provides previously unknown SEM eggshell information from curated species, which contributes with a specific and supplementary taxonomic feature at the species level of Capillariidae.

Keywords Scanning electron microscopy · Capillariidae · Taxonomy · Eggshell ornamentation

Introduction

Nematodes of the family Capillariidae Railliet, 1915, parasites of vertebrates, have a complex and controversial taxonomy, mostly because of lack and/or misinformation of morphology of many species. The main feature for identification of genera is based on the morphology of the posterior end of males. The most used features are the spicular sheath, spicule, structure of stichosome, bacillary bands, vulvar appendage, and, among other characteristics, the structure of eggs (Moravec 1982; Lomakin and Romashov 1987). The structure of the outer

surface of the eggshell are very diverse among capillariids and have been recognized as the most important characteristics for identification of capillariid species through eggs (Conboy 2009; Zajac and Conboy 2012; Macchioni et al. 2013).

However, studies characterizing eggs are scarce, especially in capillariids. Among all the structures used to identify species, the egg is the evolutionary form that appears in most studies, such as coprological surveys on medical, ecological, and paleoparasitological fields. The most studied species are of public health or veterinary concern, for instance *Calodium hepaticum* Bancroft, 1893, *Paracapillaria philippinensis* Chitwood, Velasquez, and Salazar, 1968, *Eucoleus aerophilus* Creplin, 1839 (Sukontason et al. 2006; Traversa et al. 2011; Macchioni et al. 2013). Romashov (1985) was a pioneer in the categorization of Capillariidae species by the ornaments of the eggshell. He proposed 6 groups according to the similarities of the ornamentations relating to the site of infection by light microscopy (LM), as well as, scanning electron microscopy (SEM) (Romashov 1985). Recently, it was proposed types of eggshell ornamentations after evaluation of

997 eggs of 28 capillariid species deposited on institutional helminthological collections using LM (Borba 2019). The study allows to develop a new methodology for capillariid species identification based on artificial intelligence technology (Borba 2019). Paleoparasitological studies, which investigate parasites in archaeological and paleontological samples, also have used eggshell ornamentations for supporting species discrimination (Fugassa et al. 2008; Araujo et al. 2013; Le Bailly et al. 2014). Coprological and paleoparasitological studies are usually conducted by LM and do not have a high support of SEM assays, since there are few species characterized (Romashov 1985; Sukontason et al. 2006; Traversa et al. 2011; Macchioni et al. 2013). Although helminth eggs have few taxonomic characters for species identification, they are the only evolutionary structure available for parasite diagnosis in coprolites or fecal samples.

In order to supply additional information for the identification of parasite species through eggs, the present study aimed to characterize eggshells from species deposited in an Institutional Helminthological Collection using SEM. Specimens from a taxonomic repository were used, since they are described and identified by systematics specialists,

Materials and methods

Twelve capillariid species of the curated Helminthological Collection of Oswaldo Cruz Institute (CHIOC) were available. Hosts, sites of infection, and voucher numbers of these species are in Table 1, and additional information about the capillariid specimens can be accessed on CHIOC online dataset (http://chioc.fiocruz.br/catalogue). The specimens were originally preserved in 70°GL ethanol and in good preservation condition. For the analysis, they were washed with phosphate buffered-saline (PBS). The final portion of the uterus of females was sectioned in order to collect fully developed eggs. Worms were broken, and their eggs extracted using an ultrasonic sonicator (Cristófoli®) for 60 s at frequency of 42 Khz.

For LM procedures, the samples were mounted in temporary slides with glycerol, examined and analyzed using a Nikon Eclipse E200 microscope. Thirty eggs of each species were measured in the × 400 magnification with the software Image Pro Plus - Media Cybernetics, USA.

The preparation for SEM was performed with the samples adhered in microscope slide cover glass (22 × 22 mm) previously prepared with porcine gelatin solution 1%. Then, samples were dehydrated in a graded ethanol series (30° to absolute), critical point dried in CO_2, mounted on stubs, coated with gold (20–25 nm) and examined using the SEM Jeol JSM-6390LV, under 15-kV acceleration voltage (Lopes Torres et al. 2013). Ten eggs were analyzed for each sample, collected from different females of the same species. Observations were conducted at different magnifications for egg overview (× 2300) and eggshell surface details (× 8000 and × 10,000).

Results

The capillariid eggs showed the characteristic barrel–shaped morphology with polar plugs and the size reported in Table 2. Eggshell surface were discriminated by LM into 4 putative groups as described in previous studies based on (1) smooth (ST), that has no ornaments on the shell, as described by Conboy in *Trichuris trichiura* eggs (Conboy 2009); (2) punctuated (PT), that has dots like a pitted surface, as described in *Eucoleus boehmi* Supperer, 1953 (Conboy 2009; Traversa et al. 2011); (3) reticulated type I (RTI) that is presented like a network as an interconnected ridges described in *Eucoleus aerophilus* (Conboy 2009); and (4) reticulated type ll (RTII) that is presented like a network but with an orientation of deep longitudinal ridges, as described in *Aonchotheca putorii* (Rudolphi, 1819) López-Neyra, 1947 (Zajac and Conboy 2012).

SEM eggshell patterns were characterized considering the arrangement of the ornamentation unit on egg surface. It was possible to observe 6 types of eggshell surface ornamentations using the SEM method (Figs. 1, 2, and 3). The SEM eggshell patterns were characterized and classified as follows.

- Type 1: The shell is overall smooth without ornamentation, as the smooth shell pattern with no ridges or pits described for *T. vulpis* (Traversa et al. 2011). In contrast, discreet longitudinal rays were observed (Fig. 1a–1b). Only one species was identified with these characteristics, *Aonchotheca pulchra* (Freitas, 1934). When LM was used, no visible ornamentation was seen (Fig. 1c), but in SEM images the mild rays appeared, specially near polar plugs (Fig. 1a).
- Type 2: The ornamentation is as a beam-like neuron-shaped matrix connected with the pillars, as defined for *Paracapillaria philippinensis* (Sukontason et al. 2006). Two species have shown this pattern: *Baruscapillaria spiculata* (Freitas, 1933) (Fig. 1d–f) and *Capillaria brasiliana* (Freitas, 1933). *Capillaria brasiliana* (Fig. 1g–i) has a less dense matrix, whereas *Baruscapillaria spiculata* has a more intricate network.
- Type 3: *Capillaria collaris* (Linstow, 1873) (Fig. 1J-L) has a very particular ornament in SEM, it is a matrix composed by a basal circular mesh with holes, which creates a very large grid when observed in LM.
- Type 4: The egg surface has a very thin tangle appearance, with a dense network with a fine mesh surrounding irregularly distributed small pits, which give the eggs a porous appearance. In *Baruscapillaria rudolphii* (Moravec, Scholz and Nasincova 1994) (Fig. 2g–i), undulation is observed as a wrinkle formed with the tangle in SEM which is reflected on

Table 1 Information of capilariid species characterized in this study

Species	Collection voucher	Locality	Host species	Host class	Infection site
Aonchotheca pulchra	CHIOC 18215	Rio de Janeiro/BR	*Tadarida laticaudata*	Mammal	Stomach mucosa
Baruscapillaria obsignata	CHIOC 26715	Bahia/BR	*Gallus gallus domesticus*	Avian	Small intestine
Baruscapillaria rudolphii	CHIOC 7770	Rio de Janeiro/BR	*Tinamus solitarius*	Avian	Small intestine
Baruscapillaria spiculata	CHIOC 2863	Rio de Janeiro/BR	*Carbo vigua*	Avian	Small intestine
Capillaria venusta	CHIOC 23408	Pará/BR	*Ramphasto toco*	Avian	Small intestine
Capillaria collaris	CHIOC 18904	Espírito Santo/BR	*Gallus gallus domesticus*	Avian	Cecum
Capillaria brasiliana	CHIOC 7046	Rio de Janeiro/BR	*Nycticorax naevius*	Avian	Small intestine
Echinocholeus hydrochoeri	CHIOC 11214	Mato Grosso/BR	*Hydrochoerus capybara*	Mammal	Small intestine
Echinocholeus auritae	CHIOC 7786	Rio de Janeiro/BR	*Metachirops opossum*	Mammal	Small intestine
Eucoleus perforans	CHIOC 9898	São Paulo/BR	*Numida meleagris*	Avian	Esophagus mucosa
Eucoleus contortus	CHIOC 6307	Rio de Janeiro/BR	*Sterna maxima*	Avian	Esophagus mucosa
Eucoleus dubius	CHIOC 7004	Rio de Janeiro/BR	*Attila cinereus*	Avian	Esophagus mucosa

CHIOC Helminthological Collection of Oswaldo Cruz Institute—"*Coleção Helmintológica do Instituto Oswaldo Cruz*". Additional information on CHIOC online dataset (http://chioc.fiocruz.br/catalogue)

LM as reticulated type I. Whereas *Echinocoleus hydrochoeri* (Travassos, 1914) (Fig. 2a–c) has flaws in the surface forming holes in SEM, but it is shown as a network in LM. *Capillaria venusta* (Freitas and Mendonca, 1958) (Fig. 2j–l) presented the same tangle pattern as type 4 when observed in close detail, but it seems to have other type of ornament in lesser magnification, which is seen in LM micrography. *Eucoleus contortus* (Creplin, 1839) (Fig. 2d–f) has a tangle network, with no tumid connections.

- Type 5: The outer surface has a solid surface with shallow pore-like appearance. Two species presented these characteristics, *Eucoleus perforans* (Kotlan and Oross, 1931) (Fig. 3a–c) that has more sparse depressions, giving a punctuated appearance in LM. Whereas *Baruscapillaria obsignata* (Madsen, 1945) (Fig. 3D-F) has a dense quantity of craters; the closeness of these craters creates a network when visualized in LM.

- Type 6: The egg ornament has different depths of asymmetrical pore-like appearance, with a dense matrix surface. Two species fit in this description, *Echinocoleus auritae* (Travassos, 1914) (Fig. 3g–i), with a more uneven shell, while *Eucoleus dubius* (Travassos, 1917) (Fig. 3k–l) has sparse pore-like appearance in SEM. Both species show a similar network in LM.

Discussion

Researches regarding capilariid shell ornamentation by LM (Romashov 1985; Fugassa et al. 2008; Le Bailly et al. 2014; Borba 2019) categorized the species by the shell surface, which is cited as the most important characteristic of eggs

Table 2 Results of capilariid eggshell ornamentation characterized in this study

| Species	LM analysis	SEM analysis	Amplitude measures (L×W) (µm)
Aonchotheca pulchra	Smooth	Type 1	46.35–52.96 × 28.15–34.40
Baruscapillaria obsignata	Reticulated type I	Type 5	47.52–51.78 × 26.67–33.45
Baruscapillaria rudolphii	Reticulated type I	Type 4	52.57–57.56 × 22.88–26.84
Baruscapillaria spiculata	Punctuated	Type 2	51.48–55.69 × 24.11–30.47
Capillaria venusta	Reticulated type II	Type 4	54.01–61.54 × 25.29–32.27
Capillaria collaris	Reticulated type I	Type 3	46.91–56.03 × 25.80–30.03
Capillaria brasiliana	Punctuated	Type 2	43.10–50.24 × 19.06–22.88
Echinocholeus hydrochoeri	Reticulated type I	Type 4	46.18–51.58 × 22.88–26.95
Echinocholeus auritae	Punctuated	Type 6	56.13–59.84 × 24.71–27.82
Eucoleus perforans	Punctuated	Type 5	37.06–42.81 × 18.15–23.94
Eucoleus contortus	Punctuated	Type 4	46.70–54.07 × 24.49–28.21
Eucoleus dubius	Reticulated type I	Type 6	47.07–55.25 × 22.40–25.57

L×W length × width
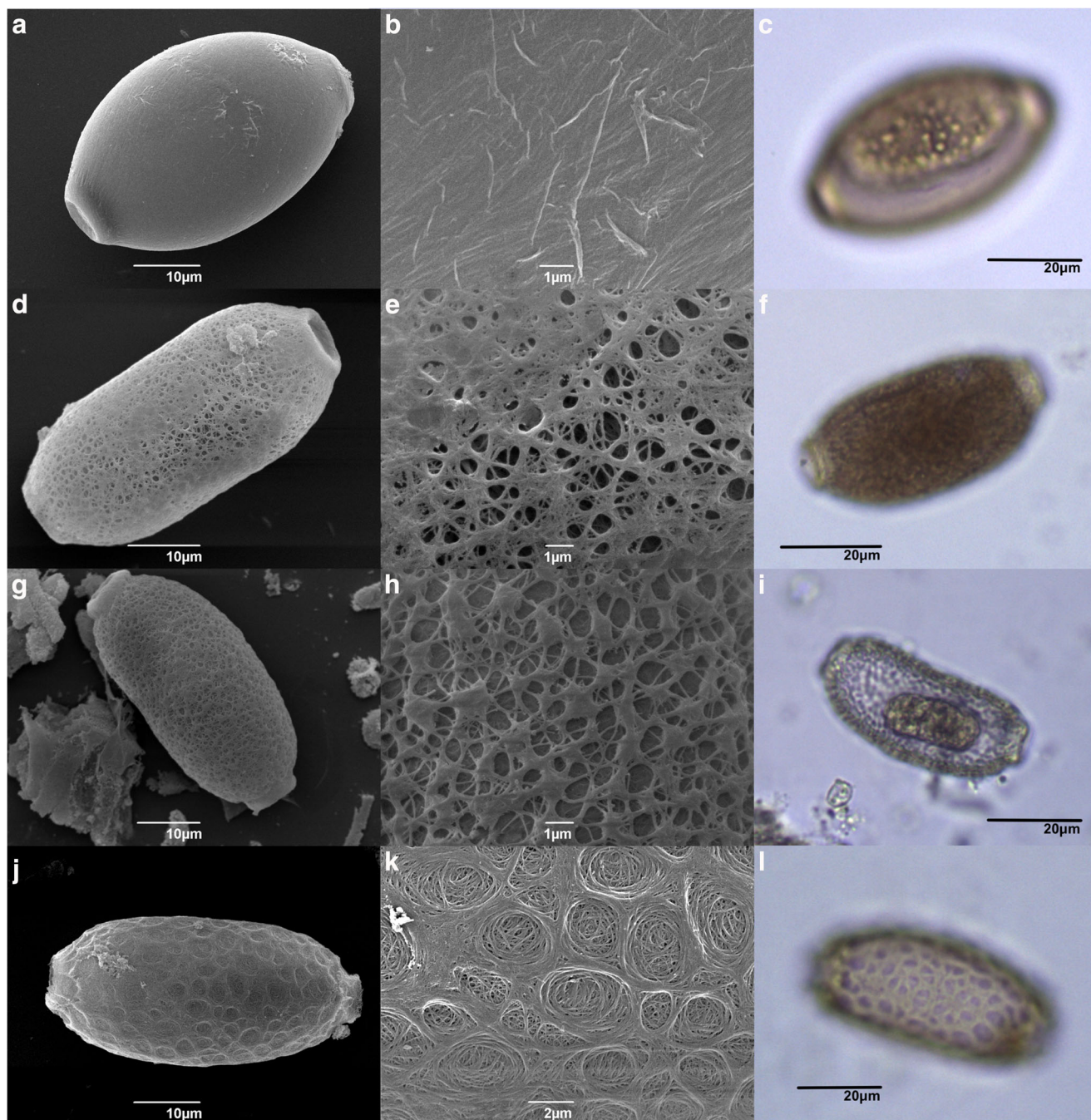
Fig. 1 SEM overview, SEM detail and LM micrographies of eggs surface of capillariids from Helminthological Collection of Oswaldo Cruz Institute (CHIOC). SEM type 1 (**a**, **b**), LM smooth type (**c**); SEM type 2 (**d**, **e**, **g**, **h**), LM punctuated type (**f**, **i**), and SEM type 3 (**j**, **k**), reticulated type I (**l**). *Aonchotheca pulchra* (**a**–**c**); *Baruscapillaria spiculata* (**d**–**f**), *Capillaria brasiliana* (**g**–**i**); *Capillaria collaris* (**j**–**l**). The LM images are intentionally blurry to focus on ornamentation

for species identification. In coprological studies, the egg is the main source of parasite identification, both in modern and archaeological samples, which use mainly the LM method (Fugassa et al. 2008; Araujo et al. 2013; Le Bailly et al. 2014). In reason of the resolution limitation of the bright field LM results, when compared to SEM images, details of the eggshell surface cannot be identified. The categorization of surface eggshell can be more informative using the SEM

method due to the resolution and image formation process, improving characterization of egg topography by high magnification and adding new features about the structural organization. Therefore, it supports the identification of the capillariid species in researches which only eggs are found.

When comparing SEM and LM classifications, we can see an accordance in type 2, the eggs in this category are both PT. On the other hand, types 4, 5, and 6 do not follow the same
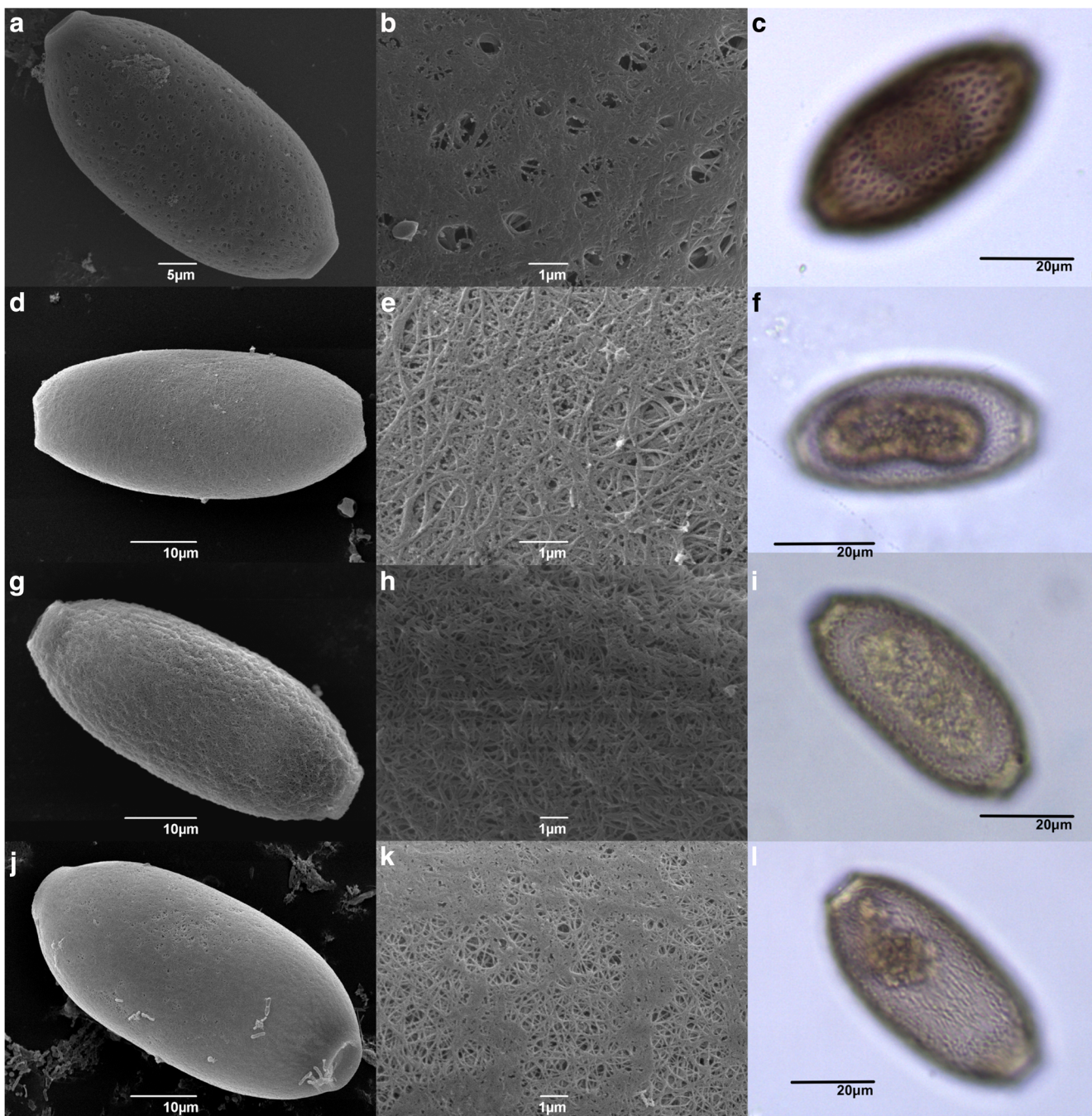
Fig. 2 SEM overview, SEM detail and LM images of eggs surface of capillariids from Helminthological Collection of Oswaldo Cruz Institute (CHIOC). SEM type 4 (**a**, **b**, **d**, **e**, **g**, **h**, **j**, **k**); LM punctuated type (**f**), LM reticulated type I (**c**, **i**), LM reticulated type II (**l**). *Echinocoleus hydrochoeri* (**a**–**c**); *Eucoleus contortus* (**d**–**f**), *Baruscapillaria rudolphii* (**g**–**i**); *Capillaria venusta* (**j**–**l**). The LM images are intentionally blurry to focus on ornamentation

accordance, as the LM patterns are PT and RT in all those types. This shows that the SEM is a fine method to characterize egg surface, complementing the results obtained using the LM.

Even though some similarities on shell ornaments can be recognized among species, no patterns are seen related to genus, site of infection, or host class. Although the ideal way to identify a worm species is based on an adult parasite through the necropsy of the animal, the egg can be a valuable stage to guide to the closest identification possible along with ecological, locality, and host data. One limitation is that eggs found in feces may be different from the eggs described in the present study. It is important to emphasize that, in the present study, in order to access capillariid specimens from curated species, with a strong taxonomic support, eggs were recovered and processed from female uterus. Therefore, they are likely
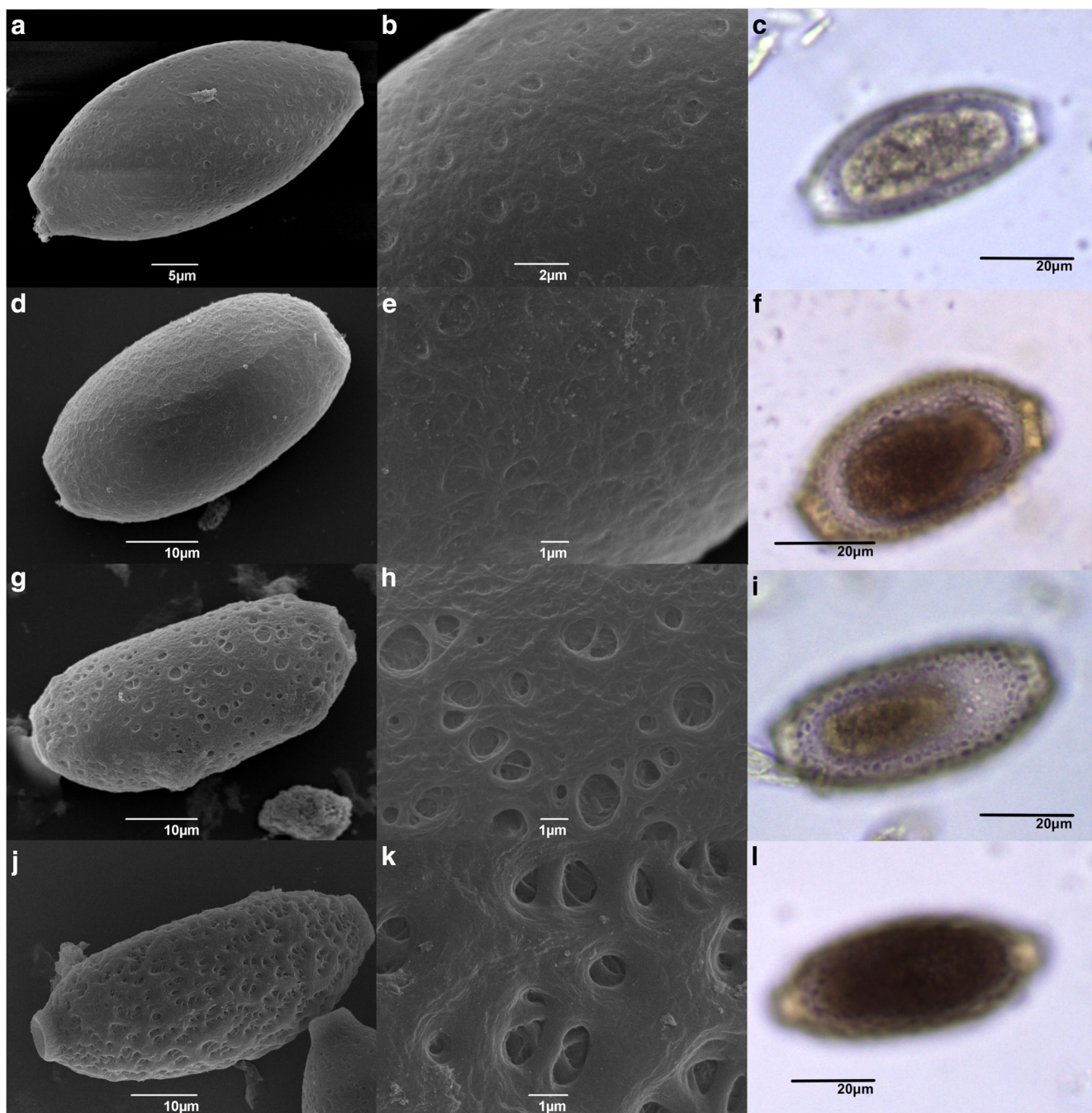
Fig. 3 SEM overview, SEM detail and LM images of eggs surface of capillariids from Helminthological Collection of Oswaldo Cruz Institute (CHIOC), SEM type 5 (**a**, **b**, **d**, **e**) and SEM type 6 (**g**, **h**, **j**, **k**); LM punctuated type I (**c**, **i**); and LM reticulated type I (**f**, **l**). *Eucoleus* *perforans* (**a**–**c**); *Baruscapillaria obsignata* (**d**–**f**), *Echinocoleus auritae* (**g**–**i**); *Eucoleus dubius* (**k**–**l**). The LM images are intentionally blurry to focus on ornamentation

not identical to those found in coprological and paleoparasitological surveys and a slightly difference in morphology and/or morphometry could be expected. It is known that helminth eggs can change during maturation outside the female. Hence, we recommend that this constrain should be taken into account when applying diagnostic samples.

The results presented here disagree with the previous study that showed 17 species from 6 genera, *Eucoleus*, *Calodium*, *Liniscus*, *Thominx*, *Capillaria*, and *Skrjabinocapillaria*, distributed into 6 categories of ornamentation Romashov ([1985](#)). The eggshell ornamentation was related to the site of infection in capillariids of mammals and has shown the same ornamentation into each genus, except for *Capillaria* spp., which was classified with two different ornamentation types (Romashov [1985](#)). We should consider that a different solution for materials fixation was used in the preceding work by Romashov,

3% formalin. In contrast, in the present work, 70°GL ethanol was applied, following the laboratory protocol. It has been evidenced that different fixation solutions have influence on morphometry. However, they have less impact on the surface topography and structural morphology (Lamberti and Sher 1969; Grewal et al. 1990). Although the fixation methodology that uses ethanol solution can promote morphometrical changes caused by dehydration, it does not have a chemical characteristic that causes alteration on the surface morphology of the eggs (Aneta Chałańska et al. 2017).

The species *Capillaria italica* and *Skrjabinocapillaria eubursata*, described by Romashov (1985), showed the same smooth surface on the eggs as type 1 (ST in LM) *Aonchotheca pulchra*. The same author described the ornaments of some species of two genera also described in the present work, *Capillaria* (*C. bovis*, *C. erinacei*, *C. minuta*, *C. petrovi*, *C. putorii*, *C. sadovskoi*) and *Eucoleus* (*E. bacillatus*, *E. hernardi*, *E. lemmi*, *E. oesophagicola*). The species of genus *Capillaria* showed the same pattern of *C. venusta,* described here as comb-like formations, which resemble small cords extending from one pole to the other. These species were classified as RTII in LM, but they do not have the same pattern in SEM.

The description for *Eucoleus boehmi* (Magi et al. 2012) comprises a delicately pitted surface with a dense network with a fine mesh, which gives the egg a porous appearance, matching with type 4 that includes *E. contortus*, *E. hydrochoeri*, *B. rudolphii*, and *C. venusta*. Although when observed in LM the characteristics of the outer shell are different in *E. hydrochoeri* and *C. venusta*, they show a reticulated type, whereas *E. contortus*, *B. rudolphii*, and *E. boehmi* have a PT pattern.

The eggs of *P. philippinensis* described by Sukontason and collaborators (2006) presented three different kinds of ornamentation: smooth, beam-like network and a combination of the two on the same egg. The eggs that were entirely intricated with beam-like network are similar to SEM type 2. Although smooth surface observed by the authors was not seen in *B. spiculata* nor in *C. brasiliana*, no egg showed the same pattern of more than one kind of surface in the present study.

Traversa and collaborators (2011) described *E. aerophilus* as a network of anastomosing ridges and bridges that resembles the eggs of type 6. Although these ridges in *E. auritae* and *E. dubius* are more subtle and give a sparse pore-like appearance instead of bridges. The pattern described for *C. hepaticum*, as irregularly distributed small pits, which give the eggs a porous appearance (Machioni et al. 2013), was not seen in any egg in the present study.

This is an initial study describing eggshell ornamentation, including new data of 12 species from more than 300 described in the family Capillariidae (Gibbons 2010). The categories proposed here are an attempt to compare the data produced by both microscopies, especially the confirmation and complementary results of patterns obtained by bright field LM and the gain of surface detail in high magnification using the SEM tool. Since the source are specimens curated by specialists and deposited in an Institutional Helminthological Collection, the main purpose was to find taxonomic features that contribute with species or genus identification by characterization of eggs. It was clear that in the same genus there are different types of texture (Table 2), excluding any idea of a pattern on eggshell to determinate a genus.

Although there is a difference in categorization by LM and SEM methods, the objectives of their use are different. As a routine method, the LM offers a rapid preparation and diagnosis, in addition to using a cheaper and accessible equipment (Beltrame et al. 2018; Beltrame et al. Sianto et al. 2014; Taglioretti et al. 2014; Agostini et al. 2018). On the other hand, SEM is a most laborious, specialized and costly methodology, mostly used as a tool for description and characterization of species (Traversa et al. 2011; Macchioni et al. 2013). Our results opened new insights on the description of the topography of Capillariidae eggs. We associated LM and SEM enabling other works using only LM to make new interpretations based on their bright field results, mainly consulting the images of this paper.

Despite not being usual to use eggs for species identification due to the lack of informative taxonomic characteristics, it is not always possible to access the adult specimens (e.g., coprological surveys, paleoparasitological studies). Therefore, a detailed egg characterization of selected curated species is important as a reference tool.

We should assume that egg ornaments probably do not have any taxonomic meaning among capilariid identification at the genus level. However, SEM morphology of capilariid eggs is a valid taxonomic element for species discrimination, at least for some species such as *E. boehmi*, *E. aerophilus*, *A. pulchra*, *B. spiculata*, *C. brasiliana*, *C. collaris*, *E. hydrochoeri*, *E. contortus*, *B. rudolphii*, *C. venusta*, *E. perforans*, *B. obsignata*, *E. auritae*, and *E. dubius*, as showed here and reported before (Magi et al. 2012). So, together with LM morphology and morphometry, SEM data is useful for the identification of the species within the Capillariidae family.

Conclusion

The SEM method is a powerful tool for eggshell characterization due to the higher magnification of surface structure. Although egg is not the main structure for species identification, as well SEM is not a tool used in routine surveys, the results give auxiliary information of capilariids not previously studied by this method. In the present study, we established categories of eggshell ornamentation in order to verify a relationship between the microscopies, and particularly, to

capillariid taxonomy. Nevertheless, the results revealed no association, but corroborated with new data to the complex taxonomy of capillariids. This study provides SEM eggshell information for selected species from taxonomic repositories, contributing to their identification. The material comes from a curated Museum collection and thus could be a good example of the use of Museum collections for modern work.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

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References

Agostini I, Vanderhoeven E, Beldomenico PM, Pfoh R, Notarnicola J (2018) First coprological survey of helminths in a wild population of black capuchin monkeys (*Sapajus nigritus*) in northeastern Argentina. Mastozool Neotropical 25:269–281. https://doi.org/10.31687/saremMN.18.25.2.0.11

Araujo A, Reinhard K, Ferreira LF, Pucu E, Chieffi PP (2013) Paleoparasitology: the origin of human parasites. Arq Neuropsiquiatr 71:722–726. https://doi.org/10.1590/0004-282X20130159

Beltrame MO, Bellusci A, Fernández FJ, Sardella NH (2018) Carnivores as zoonotic parasite reservoirs in ancient times: the case of the Epullán Chica archaeological cave (Late Holocene, northwestern Patagonia, Argentina). Archaeol Anthropol Sci 10:795–804. https://doi.org/10.1007/s12520-016-0399-8

Borba V (2019) Taxonomia Integrativa, Filogenia e Paleodistribuição de Capilarídeos. PhD thesis, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, p. 179.

Conboy G (2009) Helminth parasites of the canine and feline respiratory tract. Vet Clin North Am Small Anim Pract 39:1109–1126. https://doi.org/10.1016/j.cvsm.2009.06.006

Fugassa MH, Taglioretti V, Gonçalves ML, Araújo A, Sardella NH, Denegri GM (2008) *Capillaria* spp. eggs in Patagonian archaeological sites: statistical analysis of morphometric data. Mem Inst Oswaldo Cruz 103:104–105

Gibbons LM (2010) Keys to the Nematode parasites of vertebrates: supplementary volume. CABI.

Grewal PS, Richardson PN, Wright DJ (1990) Effects of killing, fixing and mounting methods on taxonomic characters of parthenogenetic adult female *Caenorhabditis elegans* (Nematoda: Rhabditidae). Revue de Nématologie 13(4):437–444

Lamberti F, Sher SA (1969) A comparison of preparation techniques in taxonomic studies of *Longidorus africanus* Merny. J Nematol 1(3): 193–200

Le Bailly M, Landolt M, Mauchamp L, Dufour B (2014) Intestinal parasites in First World War German soldiers from "Kilianstollen", Carspach, France. Braga ÉM, editor. PLoS ONE 9. doi:https://doi.org/10.1371/journal.pone.0109543

Lomakin V, Romashov B (1987) Morphological-taxonomical analysis and phylogenetic relationships of nematodes of the family Capillariidae Railliet, 1915. Tr Gelan 35:87–94

Lopes Torres EJ, de Souza W, Miranda K (2013) Comparative analysis of *Trichuris muris* surface using conventional, low vacuum, environmental and field emission scanning electron microscopy. Vet Parasitol 196:409–416. https://doi.org/10.1016/j.vetpar.2013.02.026

Macchioni F, Chelucci L, Guardone L, Mignone W, Prati MC, Magi M (2013) *Calodium hepaticum* (Nematoda: Capillaridae) in a red fox (*Vulpes vulpes*) in Italy with scanning electron microscopy of the eggs. Folia Parasitol (Praha) 60:102–104

Magi M, Guardone L, Prati MC, Torracca B, Macchioni F (2012) First report of *Eucoleus boehmi* (syn. *Capillaria boehmi*) in dogs in North-western Italy, with scanning electron microscopy of the eggs. Parasite 19(4):433–435

Moravec F (1982) Proposal of a new systematic arrangement of nematodes of the family Capillariidae. Folia Parasitol (Praha) 29:119–132

Romashov B (1985) Morphological peculiarities of egg shell in capillariids (Nematoda, Capillariidae). Parazitologiia 5:399–401

Sianto L, de Souza MV, Chame M, da Luz MF, Guidon N, Pessis AM (2014) Helminths in feline coprolites up to 9000 years in the Brazilian Northeast. Parasitol Int 63:851–857. https://doi.org/10.1016/j.parint.2014.08.002

Sukontason KL, Sukontason K, Piangjai S, Vogtsberger RC (2006) Ultrastructure of eggs of *Paracapillaria* (*Crossicapillaria*) *philippinensis* and evidence related to its life cycle. Micron 37:87–90. https://doi.org/10.1016/j.micron.2005.05.006

Taglioretti V, Fugassa MH, Beltrame MO, Sardella NH (2014) Biometric identification of capillariid eggs from archaeological sites in Patagonia. J Helminthol 88(2):196–202

Traversa D, Di Cesare A, Lia RP, Castagna G, Meloni S, Heine J (2011) New insights into morphological and biological features of *Capillaria aerophila* (Trichocephalida, Trichuridae). Parasitol Res 109:97–104. https://doi.org/10.1007/s00436-011-2406-4

Zajac A, Conboy GA (2012) Veterinary clinical parasitology, 8th edn. Wiley-Blackwell, Chichester, West Sussex, UK

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