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The morphology of the eggs in the *Hermanella* complex (Ephemeroptera: Leptophlebiidae)

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ABSTRACT

The *Hermanella* complex is a well-established monophyletic group of Hagenulinae mayflies, but the phylogenetic relationships among its members are still being discussed. In order to fill some gaps in the taxonomy of this group we describe, for the first time, the eggs of eight species of this complex (*Hermanella amere*, *Hermanella mazama*, *Hydromastodon sallesi, Hydrosmilodon gilliesae*, *Hydrosmilodon plagatus*, *Leentvaaria palpalis*, *Paramaka incognita*, *Paramaka* sp.) using scanning electron microscopy, including some important members whose generic allocation have been questioned. The egg morphology in these eight species of *Hermanella* complex and their similarities with other species of the complex are discussed.

Introduction

The mayflies of the *Hermanella* complex, or Hermanellognatha, form a monophyletic clade of Hagenulinae (Ephemeroptera, Leptophlebiidae) with nymphs showing specialized filtering mouthparts, such as enlarged labrum, and rows of long setae on maxillae and labium (Domínguez and Flowers, 1989; Kluge, 2007). These insects have a wide distribution in the Neotropical region, with some representatives extending to the Nearctic region (Flowers and Domínguez, 1991).

The *Hermanella* complex was defined by Domínguez and Flowers (1989) and since then new genera and several new species have been described in this group. To date, approximately 40 species belong to this complex, including the genera *Hermanella* Needham & Murphy, 1924, *Hydromastodon* Polegatto & Batista, 2007, *Hydrosmilodon* Flowers & Domínguez, 1992, *Hylister* Domínguez & Flowers, 1989, *Leentvaaria* Demoulin, 1966, *Needhamella* Domínguez & Flowers, 1989, *Paramaka* Savage & Domínguez, 1992, and *Traverella* Edmunds, 1948.

Although taxonomic and phylogenetic studies about this group have increased considerably (e.g. Domínguez et al., 2001; Domínguez and Cuezzo, 2002; Sartori, 2005; Lima et al., 2012), some relationships are poorly understood. The monophyly of *Hydrosmilodon* was discussed by Sartori (2005), whereas Kluge (2007) suggest an unique genus, *Hermanella*, with five subgenera, with *Hydrosmilodon* and *Paramaka* as junior synonyms of *Needhamella* (a view not followed by Nascimento and Salles, 2013). Monjardim et al. (2020) have also found *Hydrosmilodon* as polyphyletic and, surprisingly, *Hydromastodon* was not recovered together with other genera of the *Hermanella* complex.

In a cladistic analysis performed by Domínguez and Cuezzo (2002), based on 5 genera and 11 OTU's (operational taxonomic units), egg characters were included in order to test their value to the analysis. The genera *Leentvaaria* and *Paramaka* were not included, while *Hydromastodon* was not described by that time. In this study, the monophyly of the *Hermanella* complex was supported with new synapomorphies, including chorion sculptures, showing that egg characters are important in phylogenetic studies for the group.

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Considering that egg features are useful to understand the phylogeny of the *Hermanella* complex, data from additional species and genera may be useful to fill some gaps in future cladistics analysis. This study describes the chorion sculpture in the eggs of eight species from the *Hermanella* complex belonging to five genera, including *Leentvaaria*, *Paramaka,* and *Hydromastodon*.

Materials & Methods

The insects (preserved in 80% ethanol) were obtained from collection kept in the Museu de Entomologia da Universidade Federal de Viçosa (UFVB), Brazil and identified with aid of taxonomic keys and comparison with original descriptions (Polegatto and Batista, 2007; Lima et al., 2012; Nascimento and Salles, 2013; Domínguez et al., 2014; Salles et al., 2016). The eggs were removed from the abdomen of mature nymphs or adult females of eight species from five genera. The eggs were transferred to 90% ethanol for 15 min, hexamethyldisilazane for 10 min and air dried. Then the samples were fixed onto aluminum stubs, gold covered (20 nm thickness) and analyzed with a LEO VP1430 Scanning Electron Microscope at 15 kV in Núcleo de Microscopia e Microanálise, Universidade Federal de Viçosa (UFV). Terminology used for egg descriptions follows Koss and Edmunds (1974).

Results

Hermanella amere Nascimento & Salles, 2013 (Figs. 1a, 1b).

Egg size: 138–143 µm in length, 50–100 µm in width. General shape prismatic (Fig. 1a), pentagonal in cross section (Fig. 1b). Both polar regions concave. Chorion surface smooth or with small granules (Figs. 1a). Several knob-terminated coiled threads (KCT) in the concave polar region (Fig. 1b). Micropyle not visible.

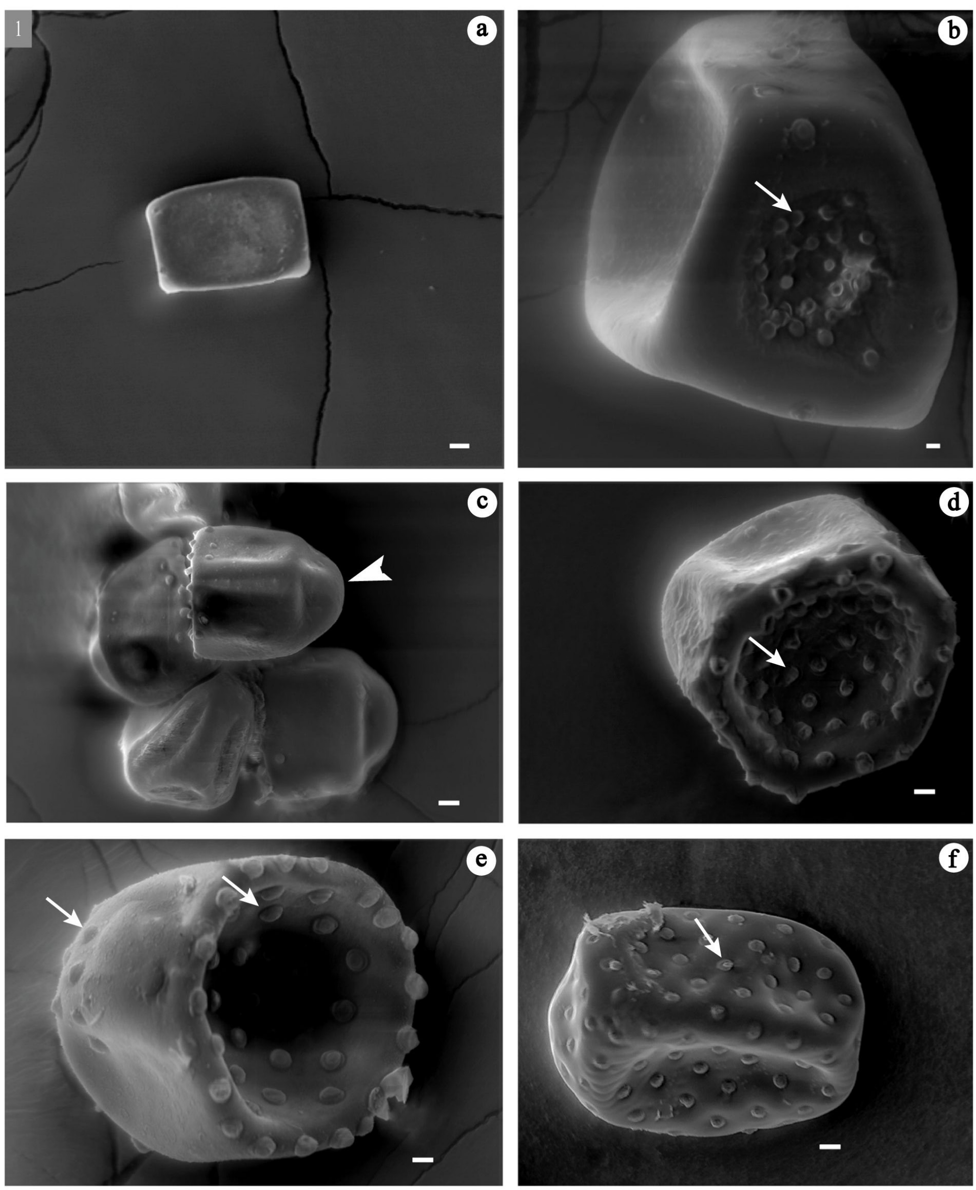

Figure 1 *Hermanella amere* **a:** General shape of egg. **b:** Knob-terminated coiled threads (arrow) concentrated only on the concave polar region. *Hermanella mazama* **c:** Chorionic surface smooth and general shape prismatic of the egg with concave and convex (arrowhead) polar region. **d:** Knob-terminated coiled threads (arrow) restricted to the concave polar region. *Hydromastodon sallesi* **e:** General shape, with knob-terminated coiled threads (arrows) distributed ring-like in the middle egg region and regularly distributed on the concave polar region. *Hydrosmilodon gilliesae* **f:** General shape of egg with knob-terminated coiled threads (arrow) regularly distributed on the surface. Scale bars: 10 µm.

Material examined. Imago: Brazil, Espírito Santo State, Ibitirama, Cachoeira do Firmino, Rio Santa Clara, 20º 35' 04" S / 41º 38' 32" W, 720 m, 04.iv.2016, light trap, Ceunes col.

Hermanella mazama (Nascimento, Mariano & Salles, 2012) in Lima et al. (2012) (Figs. 1c, 1d).

Egg size: 124–127 µm in length, 55–115 µm in width. General shape prismatic with a concave polar region (Fig. 1c), hexagonal in cross section (Fig. 1d), one concave and the other convex with some KCTs (Fig. 1c, 1d). Chorion surface smooth. Micropyle not visible.

Material examined. Subimago: Brazil, Espírito Santo State, Nova Venécia, Patrimônio do Bis, Rio Cotaxé, 18º 33' 27.5" S / 40º 20' 06.5" W, 20-21.xi.2012, pensilvania trap, E.M. Rozário, M.C. Gonçalves and K.B. Angeli cols.

Hydromastodon sallesi Pollegato & Batista, 2007 (Fig. 1e).

Egg size: 120–136 µm in length, 67–124 µm in width. General shape prismatic, with one polar region convex, the other concave (Fig. 1e). Chorion surface smooth or with very fine small granules. KCTs distributed along a ring at the equatorial region and scattered in the on polar concave surface (Fig. 1e). Micropyle not visible.

Material examined. Nymph: Brazil, Rondônia State, Nova Londrina, Rio Urupá, 11º 02' 05" N / 62º 08' 34" W, 182 m, 02.ix.2012, N. Hamada col.

Hydrosmilodon gilliesae Thomas & Péru, 2004 in Thomas et al. (2004) (Fig. 1f).

Egg size: 83–105 µm in length, 67–124 µm in width. General shape prismatic (Fig. 1f), quadrangular in cross section. Both polar regions flattened (Fig. 1f). Chorion surface smooth or with very fine small granules and KCTs regularly distributed on whole egg surface. Micropyle not visible.

Material examined. Imago: Brazil, Roraima State, Caracaraí, Cachoeira Bem Querer, 01° 55' 48.3" N / 61° 00' 09.2" W, 71 m, 14.iii.2014, light trap, F.F. Salles col.

Hydrosmilodon plagatus Lima, Nascimento & Salles, 2012 in Lima et al. (2012) (Figs. 2a-c).

Egg size: 195–200 µm in length, 125–135 µm in width. General shape oval, triangular in cross section (Figs. 2a, 2b). Polar regions flattened. Chorion surface with longitudinal costae and KCTs restricted to the polar regions (Figs. 2b, 2c). Circular area *circa* 35 µm in diameter delimited by a narrow KCTs collar (Fig. 2b). Two micropyle in the equatorial area (Fig. 2a). Internal diameter of the micropyle 3–4 µm. Circular opening micropyle smooth (Fig. 2a).

Material examined. Subimago: Brazil, Espírito Santo State, Nova Venécia, Rio Cricaré, 18° 39' 51.4" S / 40° 30' 44.9" W, 74 m, 18-19.ii.2013, pensilvania trap, E.M. Rozário, M.C. Gonçalves and K.B. Angeli cols.

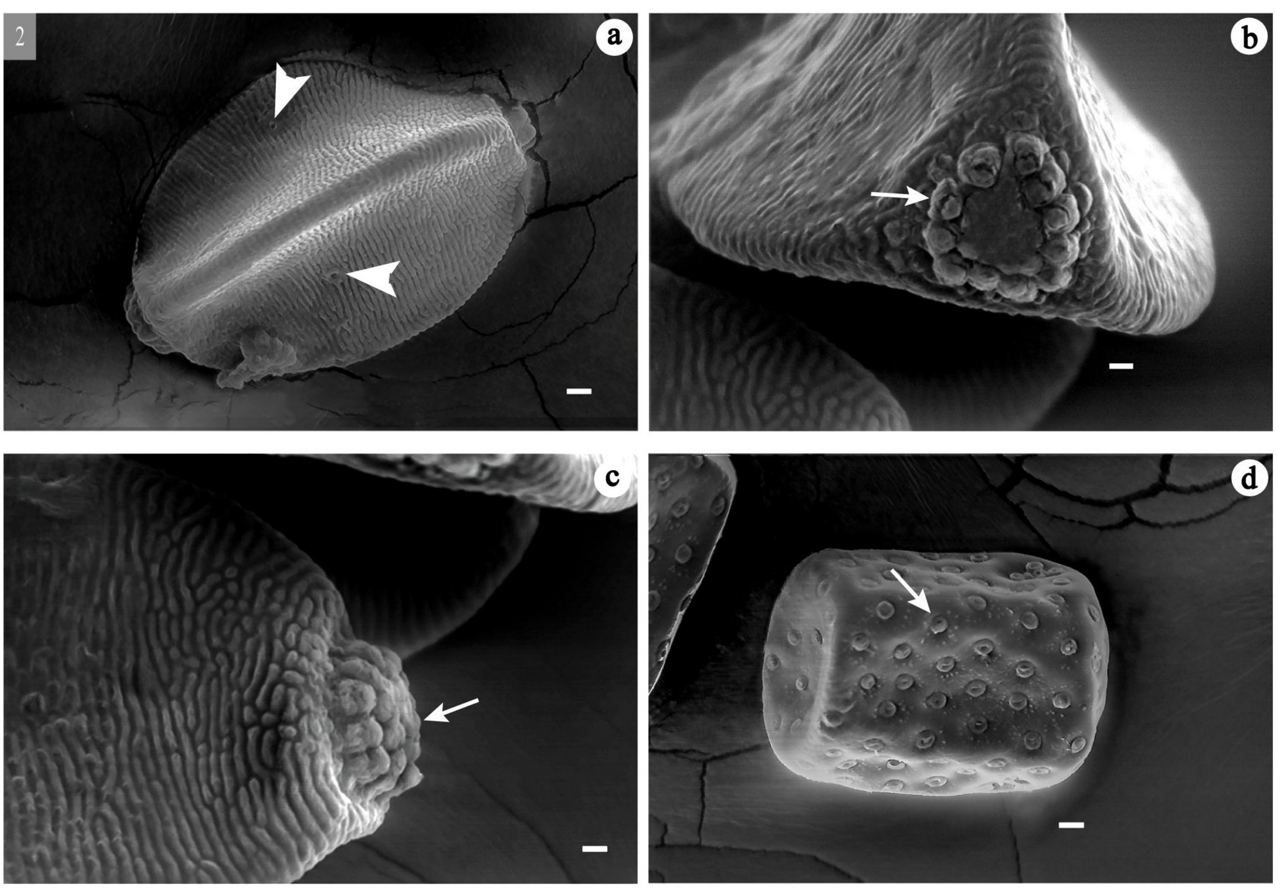

Figure 2 *Hydrosmilodon plagatus* **a:** General shape of egg with micropyles (arrowheads). **b:** Circular area *circa* 35 µm in diameter delimited by a narrow knob-terminated coiled threads collar (arrow). **c:** Knob-terminated coiled threads (arrow) restricted to the polar regions. *Leentvaaria palpalis* **d:** General shape of egg and knob-terminated coiled threads (arrow) regularly distributed on the whole surface. Scale bars: 10 µm.

Leentvaaria palpalis Demoulin, 1966 (Fig. 2d).

Egg size: 90–100 µm in length, 63–77 µm in width. General shape prismatic (Fig. 2d), pentagonal to hexagonal in cross section. Polar and lateral regions concave (Fig. 2d). Chorion surface smooth or with very fine small granules. KCTs regularly distributed on whole egg surface, granulated. Each KCT rounded with wrinkles (Fig. 2d). Micropyle not visible.

Material examined. Imago: Brazil, Roraima State, Caracaraí, Cachoeira Bem Querer, 01° 55' 48,3" N / 61° 00' 09.2" W, 71 m, 09.ii.2007, light trap.

Paramaka incognita Domínguez, Grillet, Nieto, Molineri & Guerrero, 2014 (Figs. 3a, 3b).

Egg size: 110–115 µm in length, 96–115 µm in width. General shape prismatic, circular to hexagonal in cross section (Fig. 3a). One polar region convex, the other concave. Chorion surface smooth. KCTs distributed along a ring at the equatorial region (Fig. 3a) and regularly distributed on concave polar region (Fig. 3b). Micropyle not visible.

Material examined. Imago: Brazil, Roraima State, Pacaraíma, Igarapé Miangue, 04.xi.2016, F.F. Salles and R. Boldrini, cols.

Paramaka sp. (Figs. 3c–d).

Egg size: 123–133 µm in length, 96–124 µm in width. General shape prismatic, circular to hexagonal in cross section (Figs. 3c, 3d). One polar region convex, the other concave. Chorion surface smooth. KCTs in the concave polar region (Fig. 3d). Circular area *circa* 110 µm in diameter delimited by a narrow, continuous, irregular KCT collar (Fig. 3c). Micropyle not visible.

Material examined. Nymph: French Guiana, Takaritante, 04º 37' 18.6" N / 52º 55' 38.2", 35 m, 01-04.xi.2016, E. Domínguez and S. Clavier, cols.

Discussion

The general morphology of the eggs of the *Hermanella* complex was initially investigated by Domínguez and Flowers (1989), and since then some characteristics have been often encountered, such as polar regions flattened or slightly concave, terminal knobs of KCTs elevated above coiled thread, and a supraequatorial micropyle (Domínguez and Cuezzo 2002). The most of these characteristics are also present in the species here analyzed.

The eggs of *He. amere* and *He. mazama* with KCTs restricted to the polar egg region are similar to those found in *Hermanella thelma* Needham & Murphy and *Hermanella froehlichi* Ferreira & Domínguez (Figs. 1b and 3a in Domínguez and Cuezzo 2002). However, this feature is not exclusive for the genus because it also occurs in *Hylister plaumanni* Domínguez & Flowers (Domínguez and Flowers 1989), *Paramaka* and *Hydromastodon* (present study).

The general pattern of the eggs of *L. palpalis* with KCTs regularly distributed on the whole egg surface and prismatic shape are also found in species of other genera, for example *He. guttata* Domínguez & Flowers, *N. ehrhardti* (Ulmer), *Hs. saltensis* Flowers & Domínguez (Domínguez and Cuezzo 2002), and *Hs. gilliesae* (present study). In phylogenetic studies *Leentvaaria* has been claimed as the sister group

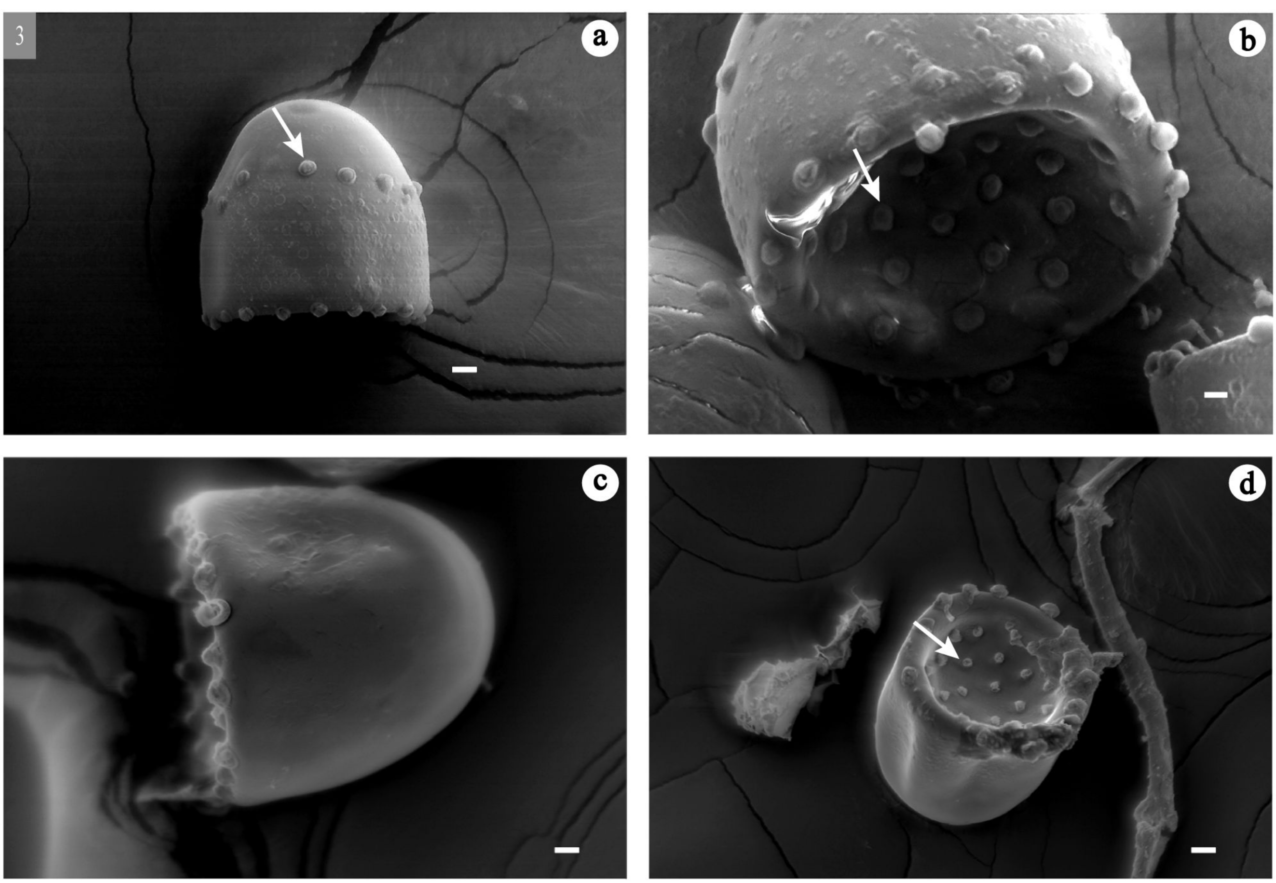

Figure 3 *Paramaka incognita* **a:** General shape of egg with knob-terminated coiled threads (arrow) ring-like in the middle egg region. **b:** Knob-terminated coiled threads (arrow) regularly distributed to the concave polar region. *Paramaka* sp. **c:** General shape and chorionic surface smooth of the egg. **d:** Knob-terminated coiled threads (arrow) restricted to the polar regions. Scale bars: 10 µm.

of *Needhamella ehrhardti* (Domínguez et al., 2001) or *Hs. gilliesae* (Sartori, 2005). Salles et al. (2016) stated that some characteristics of the imago of *L. palpalis* are also found in *Hs. gilliesae*, suggesting that they are probably closely related, which is also suggested by the similar eggs features found here.

The chorion sculpture in the eggs of *Hs. gilliesae* is also similar to that found in *Hs. saltensis*, except the occurrence of granulated bumps in the later (Domínguez and Cuezzo, 2002). The egg morphology of *Hs. plagatus* is different from the general pattern found in other *Hydrosmilodon* species, and even in the *Hermanella* complex, what gives additional evidence to the non-monophyletic nature of *Hydrosmilodon* (see Salles et al., 2016).

We also presented the first egg description for *Paramaka* (*P.* sp. and *P. incognita*), and for *Hydromastodon* (*Hm. sallesi*). The eggs of these species are similar, with circular-shape, hexagonal in cross section, one polar region concave with KCTs and the other convex, smooth. *Paramaka incognita* and *Hm. sallesi* have also KCT distributed along an equatorial ring, a characteristic not found in any other representative of the *Hermanella* complex. Further studies are still necessary to verify if these are closely related genera.

This study describes for the first time the eggs of *Leentvaaria, Paramaka, Hydromastodon* and of additional species in *Hydrosmilodon* and *Hermanella.* Overall, our findings provide new data with potential to enhance the knowledge about the systematics of the *Hermanella* complex.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

TSR conceived the research, made the egg descriptions, improve the images and wrote the document. JLAM supported the egg descriptions and helped the work in the laboratory. DSF made the plates, supported the laboratory work and made the measures of the eggs. MCG helped to describe the eggs. JES facilitated the optic equipment for took the SEM micrographs, improved the eggs descriptions and the final version of this paper. FFS improved the introduction and discussion of the document. All authors critically reviewed the manuscript and accepted the final version of this paper.

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