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SHORT COMMUNICATION

The chorion of eggs in a Namibian *Ariadna* species (Araneae: Segestriidae): morphological and SEM analyses

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Abstract. Morphological and SEM analyses were carried out on the chorion of freshly laid eggs, eggs at different time intervals after oviposition and after hatching of a Namibian segestriid spider *Ariadna* sp. The eggs laid in the laboratory are held together by a milky-white mucous secretion that gradually decreases until it almost entirely disappears. The eggs are spherical/ellipsoid in shape and, only after the reduction of the secretion, are granular structures of the exochorion evident. Granules are arranged in a single layer and lie on a compact endochorion covering the thin vitelline membrane. No significant difference was found in the chorion of hatched eggs compared to eggs a few hours after oviposition.

Keywords: Spiders, ultrastructure, egg envelopes, chorion granules

Segestriid spiders, once included in the family Dysderidae, are haplogyne araneomorphs known as tube-dwelling spiders because they permanently live inside tube-shaped retreats (Dippenaar-Schoeman & Jocqué 1997). These spiders can be considered ubiquitous as they are present on all continents, except Antarctica, and in very different habitats ranging from humid forest to arid or semi-arid environments (World Spider Catalog 2015).

Among Segestriidae, the cosmopolitan genus *Ariadna* Audouin 1826 is well known for building silk-lined tubes as nests where spiders spend essentially all their lives. Mating takes place inside the female mating tubes, eggs are laid without being enclosed in an egg sac, as is known for all Segestriidae (Eiseman & Charney 2010), and spiderlings remain inside the maternal nest for some time after hatching (Beatty 1970). In the Namibian gravel plains, Costa et al. (1993) discovered various populations of undescribed *Ariadna* species that dig an individual silk-lined burrow in the soil with a circular entrance surrounded by a ring of small stones (Costa et al. 1995; Conti et al. 2004). The silk threads placed under the stones enable the predator, waiting at the bottom of its burrow, to detect prey brushing against them (Henschel 1995). The features of the burrow rings vary according to population and habitat (Conti et al. 2015). The Namibian *Ariadna* species are adapted to live in dry habitats and, staying permanently in their burrow, have to face many difficult problems such as maintaining adequate moisture, defending against excessive heat due to soil overheating, and protecting both eggs and offspring.

While we can document some aspects of behavioral patterns of these Namibian species, (Costa et al. 1993, 1995; Costa & Conti 2013), some chemical features of silk (Conti et al. 2015), and even internal female genitalia (Michalik, Conti and Lombardo, unpublished), very little information about their life cycle, mating, egg deposition, and both embryonic and post-embryonic development is available. Here, we investigated the egg morphology of these arid-adapted spiders for the first time by means of SEM and light microscopic analyses on eggs laid in the laboratory.

From 25 to 27 March 2012, 30 adult female spiders were collected from a population of a Namibian *Ariadna* sp., in a savannah dry area of the western part of Namibia (20°25'53.1'' S, 14°20'44.9'' E). Because females of the Namibian *Ariadna* species lay their eggs in silk-lined tubes, our specimens were individually put in Falcon 50 ml conical tubes (30 x 115 mm) containing top soil from the sampling site. Then they were kept in the laboratory under the following conditions: temperature 20–25°C; approximately 25–30% RH; photoperiod similar to the sampling site; fed on a diet of insect prey. After about two to three weeks from the collection date, some females laid a clutch of eggs at the bottom of the tubes in which they were kept.

Numerous eggs from several ovipositions were examined using an optical microscope (OM) and a Scanning Electron Microscope (SEM). For OM investigation, eggs taken at different time intervals after oviposition were observed *in toto*, without pre-treatment. For SEM investigations, eggs were analyzed at different time intervals after oviposition (freshly laid eggs, 2 hrs after oviposition, $>$ 2 hrs after oviposition) and after hatching in order to highlight possible changes in the chorion. Eggs were fixed in 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer at pH 7.4 for 4 h. Then they were washed several times in the same buffer, post-fixed with 2% osmium tetroxide for 1 h, dehydrated in ethyl alcohol, immersed in hexamethyldisilazane (HMDS) (Nation 1983) and air dried. Finally, eggs were mounted on SEM stubs, metal coated, and then observed with an Hitachi S4000 microscope.

Spider females lay clutches of 30–60 eggs (Fig. 1A, arrowhead), not enclosed in an egg sac. Eggs of a single clutch are held together by a milky-white mucous secretion that gradually decreases in thickness until it almost entirely disappears two hours after oviposition. Following the decrease of the mucous secretion, eggs remain joined together by residues of the same secretion mixed with sand and thin silk threads (Fig. 1B, arrowheads). Each egg, about 1.5–1.7 mm in diameter, is whitish/ivory and spherical/slightly ellipsoid in shape (Fig. 1C). Its surface shows no evident sculptures but only small granules which become more evident within a few hours after oviposition. SEM observations show that each freshly laid egg is enveloped by the aforementioned milk-white mucous secretion of non-uniform thickness, covering, more or less completely, the chorion granules (exochorion) (Fig. 1D).

About two hours after oviposition, the mucous secretion is significantly reduced highlighting the granular structures and thin silk threads (Fig. 1E, white arrows). The granules, each approximately 1.8 µm in diameter, are loosely spaced and arranged mostly in a single layer; in some eggs, they form irregularly arranged aggregates without any order and in more layers. The number and distribution of these aggregates differ among the eggs (Fig. 1F, G).

Sections of eggs show that the chorion is made up of granules of the exochorion lying on a compact lamina (endochorion) about 0.8 µm

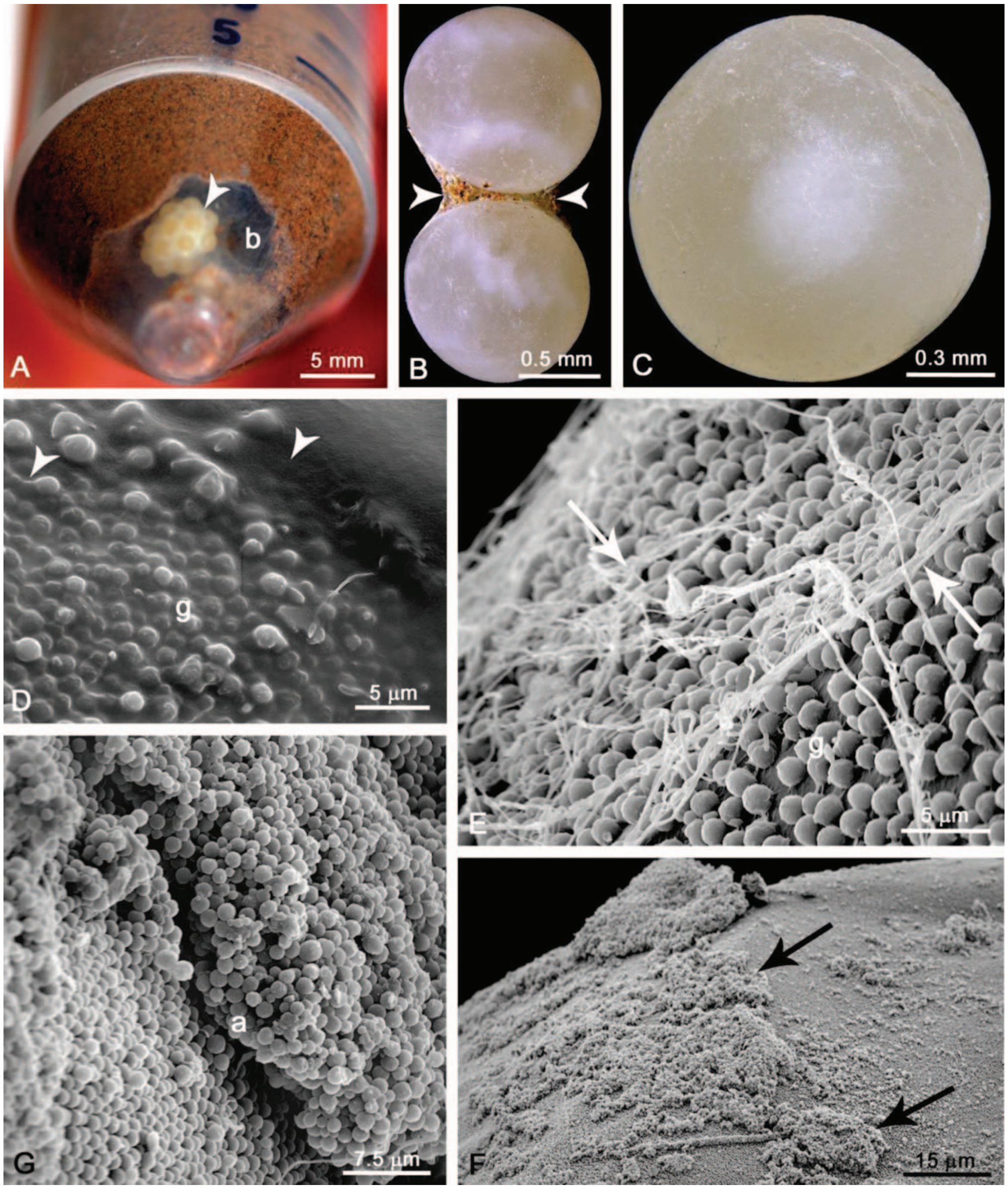

Figure 1.—OM observations of laid eggs of *Ariadna* sp. (A–C); SEM observations of chorion surface in eggs, freshly laid (D) and about 2 hours after oviposition (E–G). A. Freshly laid clutch of eggs in a burrow (b), at the bottom of a tube. A milky-white mucous secretion (arrowhead) is visible. B. Two eggs from a clutch, a few hours after oviposition, joined together by residues of secretion (arrowheads) mixed with sand and thin silk threads. C. Whitish/ivory egg from a clutch a few hours after oviposition. D. Secretion (arrowheads), more or less completely covering the granular structures of the exochorion (g). E. Silk strands (white arrows) adhering to the granular structures (g). F. Areas (black arrows) of various sizes with aggregates of the granular structures. G. Aggregate of the granular structures (a), arranged without any order and in several layers.

thick (Fig. 2A); the endochorion covers the vitelline membrane about 0.2 µm thick (Fig. 2B, C). The vitelline membrane shows barely visible hollows, uniformly arranged in freshly laid eggs (Fig. 2C, D, white arrowheads); in the latter, moreover, isolated spermatozoa can be found sticking on this membrane (Fig. 2E). In more than two hour-old laid eggs, the milky-white mucous secretion is almost completely reduced, revealing the rough surface of the granules (Fig. 2F, white arrowheads). Our analysis does not point out further structural differences among eggs at different times from deposition to after hatching.

Previous studies on eggs of Araneae and other Chelicerata (Witalinski & Żuwała 1981; Witalinski 1993; Morishita et al. 2003) clearly suggest that the vitelline membrane, being a primary envelope, is formed inside the ovary, while the chorion is formed later in the genital tract. In particular, Morishita et al. (2003) have shown that the chorion granules of the eggs of the sicariid spider *Loxosceles intermedia* Mello-Leitão 1934 are produced within the oviduct, and later these granules are closely intermingled with an opalescent material inside the uterus. This is different from what happens in pseudoscorpions where the chorion granules are produced in the ovary and become deposited on the egg surface during ovulation (Jędrzejowska et al. 2013). Even if our investigations have not considered the chorion formation, we think that the route followed by the oocyte of *Ariadna* sp. can be similar to the *L. intermedia* one. On

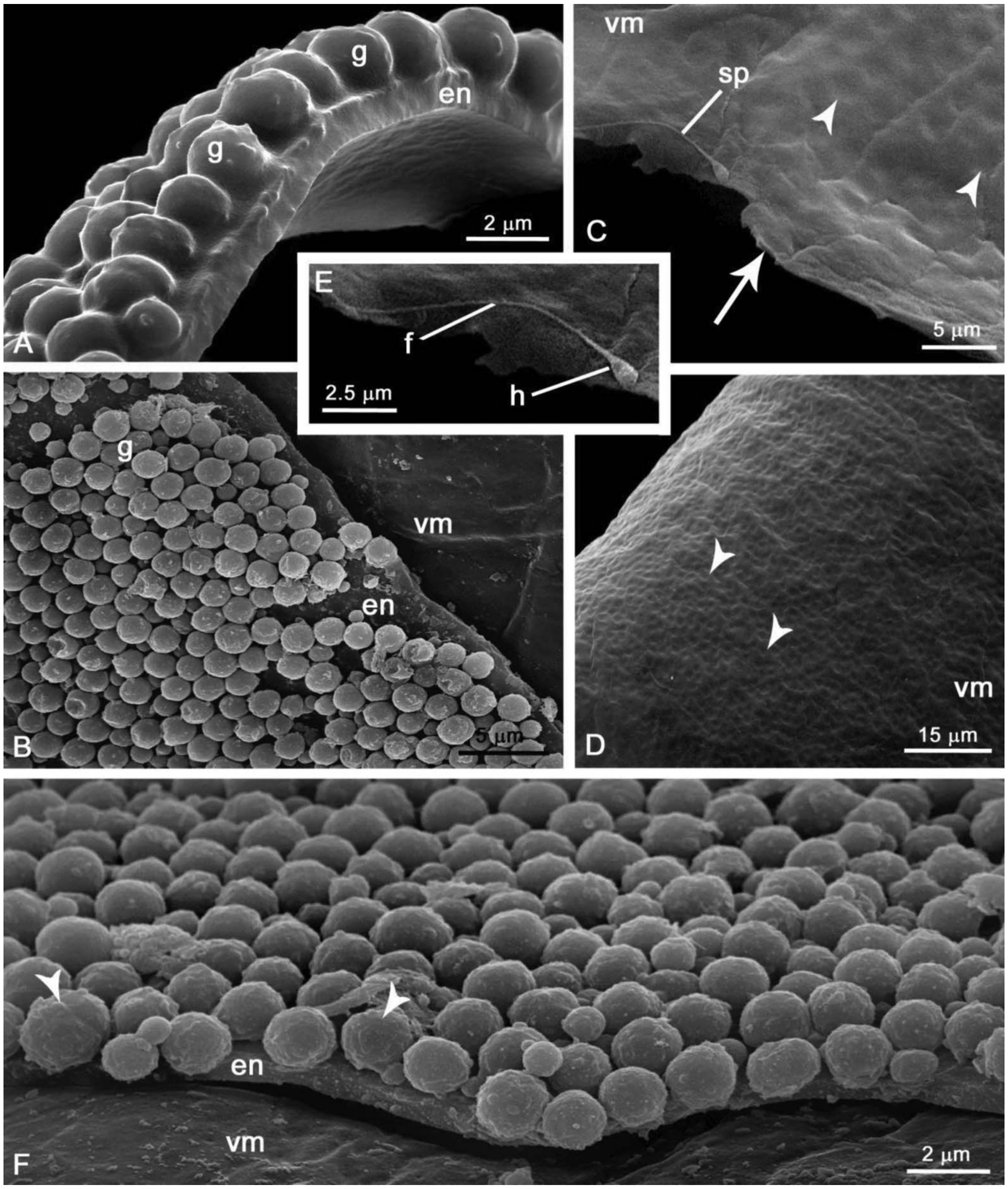

Figure 2.—SEM observations of chorion in eggs, about 2 hours after oviposition (A, B), freshly laid (C–E), more than 2 hours after oviposition (F). A. Chorion section showing the granular structures (g) of the exochorion lying on the compact endochorion (en). B. Vitelline membrane (vm) beneath the endochorion (en); granules of the exochorion (g). C & D. Barely visible hollows (arrowheads) in vitelline membrane (vm); section of vitelline membrane showing its thinness (arrow); isolated spermatozoon sticking on the vitelline membrane (sp). E. Magnification of the isolated spermatozoon in (C), showing its flagellum (f) and head (h). F. Rough surface of the granular structures (arrowheads); endochorion (en); vitelline membrane (vm).

the other hand, the production of granular chorion in the uterus has been reported in an antrodiaetiid spider (Michalik et al. 2005).

Because the average size of the numerous little hollows on the vitelline membrane is correlated to that of the granules of the exochorion, it is possible that these hollows are the marks of these structures. We cannot rule out, however, that these hollows coincide with the footprints of some ovarian cells.

The granules of the exochorion have already been found in the chorion of different species of Araneae (Humphreys 1983, 1987; Morishita et al. 2003; Michalik et al. 2005; Okada et al. 2009). However, unlike the findings in these studies, the chorion granules found in *Ariadna* sp., largely similar in size, are arranged in a single layer. In accordance with Humphreys' statement on other spider species (Humphreys 1983), the size of the granules could represent a "fingerprint" also for *Ariadna* sp. On the contrary, unlike other species of Araneae (Okada et al. 2009), both the number and the distribution of the aggregates of granules are different in the various eggs of *Ariadna* sp. we examined and, therefore, we cannot consider them as species-specific features for our species.

Regarding the rough appearance of the granules, similar to some found in grasshoppers (De Luca & Viscuso 1974; Longo et al. 1982), we suspect that this feature is linked to the function of water absorption, required for embryonic development, as already suggested for the studied orthopteran species.

The compact endochorion of *Ariadna* sp., beneath the granular structures of the exochorion, likely performs a mechanical function and it is probable that this layer also prevents egg dehydration. Furthermore, in the freshly laid eggs, the vitelline membrane underlying the endochorion would be more appropriately called the ''fertilization membrane'' because fertilization in *Ariadna* sp. is internal, as in other species of Araneae (Michalik et al. 2005; Burger & Kropf 2007). Moreover, the silk threads on the granules of the exochorion of an egg of *Ariadna* sp. could ensure adhesion of the same granules on the endochorion; in other species of Araneae, instead, there is a fine network connecting the granules that causes their adhesion to the chorion (Humphreys 1983).

The milky-white mucous secretion, which covers the chorion of the freshly laid eggs and decreases with time, could be produced by the female during the oviposition. The presence of a mucous material, produced by the female genital tracts, was also reported for other arthropods: in some grasshoppers (Longo et al. 1982; Viscuso et al. 1984) and in some phasmids (Masetti et al. 1994; Viscuso et al. 1996, 1997). In fact, a mucous secretion covers the chorion during the egg transit in the oviduct and gradually decreases until it disappears. Because the chorion genesis of insects mainly occurs within the ovary, this mucous secretion would ensure the adherence of the eggs to each other and could also be useful in limiting their dehydration (Vitale & Viscuso 2015). The milky-white mucous secretion of the eggs of *Ariadna* sp., therefore, could play similar functions, thus ensuring favorable conditions for the survival of freshly laid eggs whose chorion is not yet adequately sclerified.

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