2018. Journal of Arachnology 46:35–39

The egg sac of *Benoitia lepida* (Araneae: Agelenidae): structure, placement and the function of its layers

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Abstract. We describe the remarkable egg sac of *Benoitia lepida* (O.P.-Cambridge, 1876) (Agelenidae) from the Negev Desert, Israel. It consists of four layers: (from outside) a papery envelope, an outer loose silk layer, a "dirt layer", and an inner flocculent silk layer surrounding the eggs. The dirt layer consists of loess soil, and may include stones, snail shells, or twigs from the surroundings. Some sacs hang from a silken string over the female's web sheet, attached to barrier threads or overhanging vegetation. In these "hanging sacs", the outer papery wall is shiny white and covers the inner layers completely. Other sacs ("attached sacs") are attached to a top branch of a shrub, away from the female's web. These sacs often have little or no outer papery envelope; the outer silk wrapping encloses the branch, and the sac may be dull brown in color. We studied the species at two sites; at one the hanging sac type predominated, while at the other the attached type was most common. A field experiment revealed that the dirt layer is effective in protecting the sacs against predation from ants but had no effect on spider predators of the egg sacs.

Keywords: Egg cocoon, manipulative experiment, anti-predatory function, Negev desert

Spider eggs are covered with silk, which forms an egg sac (Foelix 2011). Most egg sacs have a layer of flocculent silk surrounding the eggs. Apart from that, the structure of the sac varies among species depending on the environment in which they are positioned and the dangers they meet from oviposition until emergence of the young (Austin 1985). Suspension in the air on silken strings, strong paper-like surfaces, and other elaborate structures have evolved presumably to protect against egg predators and parasitoids as well as fluctuating climatic conditions (Foelix 2011). Experimental studies have confirmed the anti-predatory function of egg sac structures, while their role in protection against the climate may be minor if any (Christenson & Wenzl 1980; Austin 1985; Hieber 1985, 1992a,b).

The spider *Benoitia lepida* (O. Pickard-Cambridge, 1876) was extremely common throughout the summer months in many parts of the Negev Desert, Israel, when the present studies were performed (1990–91). However, in recent years their numbers have diminished for unknown reasons, and lately it has been difficult to find them. Their funnel webs are placed at the base of shrubs (Fig. 1A). At some localities (e.g., near Sede Boqer in the Negev desert highlands, 30°51′8.27″N 34°47′0.24″E), egg sacs were seen suspended among the barrier threads over the female's web (Fig. 1B). These egg sacs were shiny white and could be observed from a long distance. By opening some of the sacs, we observed a multilayered structure, one of the layers composed of loess soil and often including stones, fragments of snail shells (Fig. 2). On a few occasions, however, we found the egg sac firmly attached to a branch at the top of the shrub (Fig. 2B), with no connection to the female's web. These sacs were usually dull in color but had the same multilayered structure. Our interest in these egg sacs was further enhanced when at a different locality at a distance of less than 50 km (Borot Loz, 30°30′48″N 34°36′32″E), we found that most females produced sacs of the attached type. These initial observations raised several questions, some of which will be dealt with here. First, what is the significance of the layers of the egg sac, in particular the layer with dirt and stones (hereafter named "dirt layer")? Does the high visibility of the sacs increase the danger of bird predation? Before dealing with these questions we describe the structure of the egg sacs and how they are positioned at our two study sites.

The hanging type of egg sac (Figs. 1B, 2A) is typically drop-shaped (length 8–10 mm, diameter 6–7 mm) and situated at the end of a silken string of variable length (from $<$ 1 cm to c. 20 cm). The string and the egg sac itself are typically shiny white and clearly observable even at 5–10 m distance. The sac is nearly always suspended from the barrier threads above the female's web. Up to five egg sacs were seen above a single web. A few very thin threads attached along the string connect it with other barrier threads or with branches of the nearby shrub. These threads keep the sac in a fixed position and prevent it from swinging in the wind and being blown into the branches.

An egg sac consists of four layers (Fig. 2). The shiny white surface is due to a thin papery envelope. Below this is a thin outer, loose layer of silk enclosing the thick dirt layer. The dirt layer may consist of loess soil particles, small stones (some up to one third the length of the egg sac; Fig. 2E), pieces of snail shells, feces of desert snails (mainly *Sphincterochila boissieri* (Charpentier) and of isopods *Hemilepistes reaumuri* (H. Milne-Edwards) (Fig. 2G)), and small twigs, all tightly packed around the inner silk layer and held together by means of silk threads. Surrounding the eggs, the inner silk layer consists of loose flocculent silk. The inner silk layer is continuous with the string, while the outer three layers are packed around both (Fig. 2H).

The attached sacs were usually attached to the side of a vertical branch near the top of the shrub, usually near the center. They were connected to the branch in two ways: by a short string at the top (like a hanging sac) and by a layer of swathing silk that surrounds both the sac and the branch (Fig. 2B). Attached sacs were usually larger and more irregular in appearance than hanging sacs because a section of the branch was included in the wrapped silk. The multilayered structure is the same, except that attached sacs were often light brown in color. This was due to a thinner outer loose silk layer and a

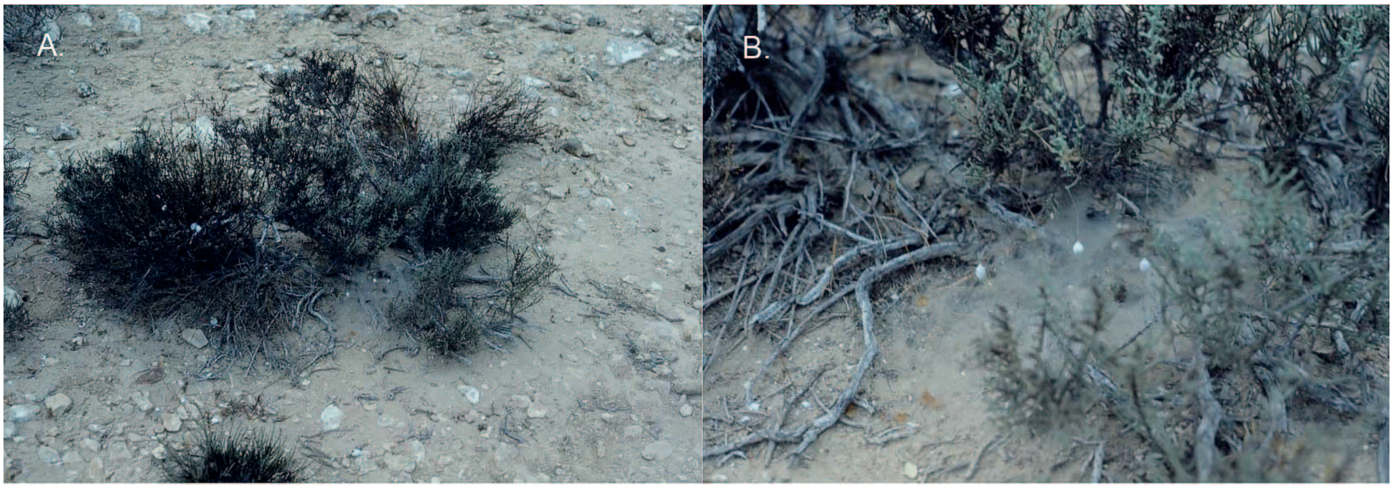

Figure 1.—A. *Benoitia lepida* web at the base of a shrub. B. egg sacs (seen as white spots) hanging above the female's web.

thinner, less papery envelope which allowed the dirt layer to be visible through it. At our Sede Boqer study site, hanging sacs constituted 97% and attached sacs 3%. At Borot Loz, the corresponding values were 24% and 76%.

We hypothesized that the peculiar egg sac structure, and in particular the dirt layer, is an adaptation against egg predators. In the field, we had observed several types of predators attacking the egg sacs: ants (*Tapinoma* sp.) and spiders (*Poecilochroa senilis* (O. Pickard-Cambridge, 1872) or *Cheiracanthium* sp.) might enter sacs and eat the eggs or young; the larvae of *Mercetina matritensis* Bolivar & Pieltain, 1934 (Hymenoptera: Chalcidoidea: Eupelmidae) develop within the egg sac and consume the eggs. To test this hypothesis, we conducted two field experiments, the first designed to examine the role of the different egg sac layers, and a second to determine if the shiny white outer layer is attractive to birds.

METHODS

Skewer experiment.—We manipulated egg sacs by removing a small piece of one or more layers from the egg sac to form a "window" on its side, judged to be a maximum of one sixth of the total surface area. The operation was performed with fine scissors under the dissection microscope.

The main experiment at the Sede Boqer site in 1991 consisted of three treatments: 1) removal of the papery + outer silk layer; 2) removal of the papery + outer silk layer +

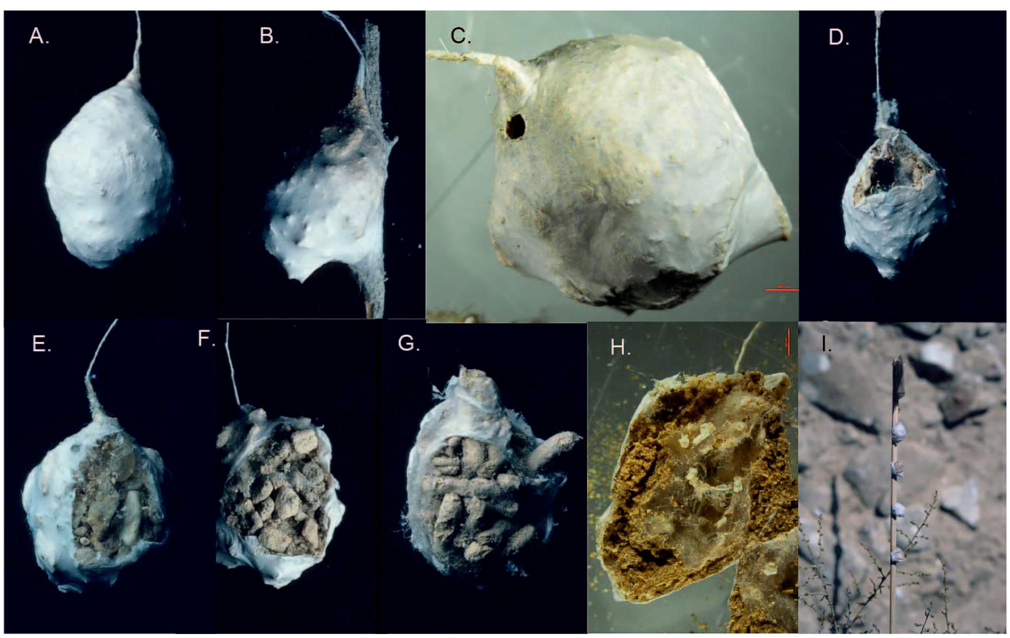

Figure 2.—*Benoitia lepida* egg sacs. A. a typical hanging sac. B. an attached sac. C. a hanging sac with exit hole from which the young have emerged. D. a hanging sac attacked by *Poecilochroa senilis*. E-F. hanging sacs with stones in dirt layer. G. hanging sac with isopod (*Hemilepistes reaumuri*) feces in dirt layer. H. hanging sac with sand grains in dirt layer; egg shells and exuviae can be seen within the inner flocculent silk layer. I. the skewer experiment: four egg sacs (3 manipulated egg sacs and a control) glued to a skewer and placed in a shrub.

the dirt layer; 3) removal of the papery+ outer silk layer + dirt layer + inner silk layer. Control sacs were sham operated, i.e., they were opened all way through to the inner silk layer and closed again. This served two purposes: first, to make sure the operation itself was not harmful to the eggs; second, to check the age of the sacs. Only sacs in which the eggs had not visibly started development were used. In this way, they were all of approximately the same age, and they would not hatch untimely.

Control and experimental sacs were glued to the side of thin wooden skewers (ca. 25 cm long) at the top end, one below another with 2–3 cm between them (Fig. 2I). The control sacs were attached such that the glue closed the sacs. The order of the treatments along the skewer was randomized and the skewers with attached sacs were positioned in low shrubs of *Artemisia sieberi* Besser, near the eastern side of each shrub (the predominant side on which *B. lepida* webs were located).

In a preliminary experiment in 1990 at Sede Boqer, only one treatment was used: removal of papery + outer silk layer + dirt layer. Thus, each skewer had a manipulated and a control sac glued to it, and all skewers were placed in a shrub on the south-facing slope. The experiment began on 10 August 1990 and the sacs were inspected four times over a period of one month.

In 1991, two experiments were performed at two sites in the Negev desert, beginning on 24 July and lasting one month, during which the egg sacs were inspected five times. One experiment with two treatment groups (removal of papery + outer silk + dirt layers and control) was performed at Borot Loz. The other experiment was performed at Sede Boqer, with a control group and all three treatment groups as described above.

The fate of the sacs was recorded when a predation effect was visible or when it had hatched. The following categories were distinguished: **sac hatched**- young presumably dispersed, no evidence of predation, the sac had a small exit hole (Fig. 2C) and the number of egg shells and exuviae from first spiderling instar inside were the same; **ant predation**- ants were present inside the egg sac and most or all eggs were consumed; **empty**- sac empty except perhaps for a few egg shells, presumed to have been preyed on by ants; **spider predation**- a large hole was visible (Fig. 2D) and some hatching may have occurred, but few spiderling exuviae were visible; and **preyed on by *M. matritensis***- the pupal case of the wasp remained inside the egg sac.

Bird predation experiment.—The visibility of the shiny white hanging egg sacs suggested that predation by birds is of no concern. We tested this assumption by means of a brief pilot experiment: in the yard behind the research institute buildings at Sede Boqer, two groups of 10 egg sacs were laid out on the ground, one group in the open, the other in the shade below trees. Flocks of house sparrows (*Passer domesticus* L.) as well as other garden birds occurred abundantly here. These birds were not likely to have had any experience with *B. lepida* egg sacs as the spider did not occur inside the village. After two days of exposure, the two plots with egg sacs were baited with bread crumbs. The egg sacs were examined after an additional two days.

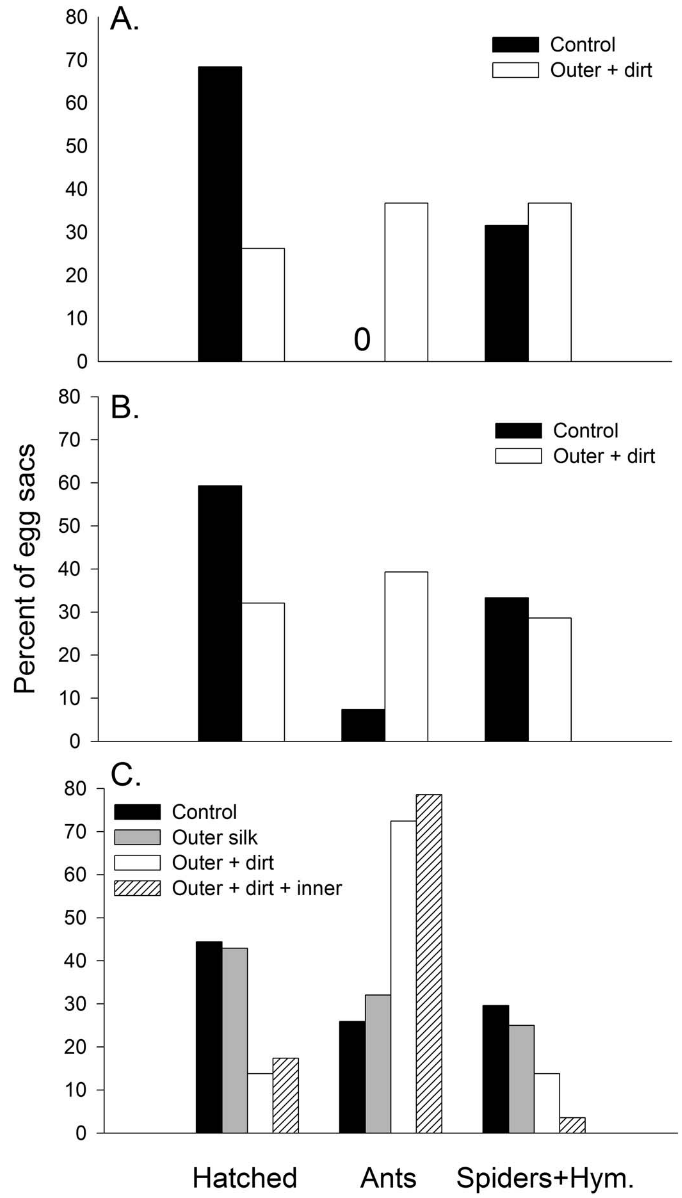

Figure 3.—Skewer experiment. Percent of egg sacs hatched, attacked by ants, or attacked by spiders or hymenopteran egg predators. A. Preliminary experiment, Sede Boqer 1990 (Control, $n = 19$; outer silk + dirt removed, $n = 19$). B. Borot Loz 1991 (Control, $n = 27$; outer silk + dirt removed, $n = 28$). C. Sede Boqer 1991 (control, $n = 27$; outer silk removed, $n = 28$; outer silk + dirt removed, $n = 29$; outer silk + dirt + inner silk removed, $n = 28$).

RESULTS

Skewer experiment.—In both the preliminary experiment in Sede Boqer (1990) and the experiment at Borot Loz (1991), significantly more manipulated sacs were attacked by ants or were emptied of eggs than control sacs ($\chi^2_2 = 10.63$, $P = 0.0053$, and $\chi^2_2 = 8.23$, $P = 0.016$, respectively; Fig. 3A,B). In both cases, there was no difference between the controls and manipulated sacs in the frequency of attacks by spiders and hymenopterans. In the second experiment at Sede Boqer, there were significant differences between the four groups ($\chi^2_6 =$

25.44, $P = 0.0005$). There was no difference between the control sacs and those from which only the papery + outer silk layer had been removed, whereas removal of the dirt layer and of the dirt layer + inner silk layer both resulted in significantly greater frequencies of attacks by ants ($\chi^2_2 = 24.15$, $P < 0.001$; Fig. 3C). Thus, it was removal of the dirt layer that made the sacs more susceptible to ant attacks, and there was no evidence that any of the silk layers contributed to the level of protection. When the outer layers + dirt were removed, seemingly there was reduced predation from spiders in the 1991 experiment (Fig. 3C); however, this may be due to the fact that a large number of the manipulated sacs (>70%) were quickly attacked by ants, and few were left intact.

Bird predation experiment.—After two days, none of the egg sacs had been touched. During the following two days, all the bread crumbs had been eaten, but still none of the egg sacs had been touched. Seemingly, they had not even been tested by the birds as they were still in the exact spots where they were laid out.

DISCUSSION

In many respects, our study is comparable to that of Hieber (1992b). He considered two orb-web spiders (Araneidae) whose egg sacs consist of multiple layers and are suspended in the air by silk threads. Hieber found that the suspension system and outer egg sac cover mainly functioned to prevent predation from ants, whereas the inner flocculent silk layer (the one directly surrounding the eggs) reduced the access for egg laying of specialized egg predators whose larvae develop in the egg sac (ichneumonids, mantispids). Similarly, we never observed ants accessing a hanging egg sac or even moving out onto the barrier threads of *B. lepida*'s web. However, even if made accessible to ants as in the skewer experiments, predation from ants on intact egg sacs was low (Fig. 3). The experiment demonstrated that the dirt layer effectively prevented access into the egg sac by ants, whereas this layer had no effect on predation from spiders.

Contrary to Hieber (1992b), we did not find any effects of the outer papery silk cover and also no effects of the inner flocculent silk layer with respect to predation risk. If they have any, more experiments focused on these layers may be needed.

In designing the pilot bird predation experiment, we assumed, following Hieber (1992b), that the barrier threads of the web were no obstacle to bird predation. From observations of many hundreds of webs, we have never seen any sign of disturbance from animals the size of birds, lizards or small mammals. Thus, we tested whether the egg sacs themselves provide any signals of being potential food to birds. Since the egg sacs were not even tested by the birds, they may rather signal not being food. The lack of interest cannot be due to negative experiences, e.g., with the dirt layer, as the birds were unlikely to have had any experience with these egg sacs before. Not all spider egg sacs are free from bird predation. Lockley & Young (1993) found a high frequency of house sparrow damage to egg sacs of *Argiope aurantia* Lucas, 1833. Barrantes et al. (2013) analyzed the light spectra emitted from the surface silk of egg sacs of spider species from several families. They concluded that reduced conspicuousness is the main function of the wavelength distribution of light reflectance (i.e., egg sac coloration). This cannot be true for the hanging sacs of *B. lepida*, which are so shiny white that conspicuousness must be intended or at least not avoided. Possibly, its high conspicuousness serves a protective function, e.g., signaling being something inedible. A test of this hypothesis should also include, however, an answer to the question why attached sacs are camouflaged and hanging sacs are conspicuous.

Ants constitute the potentially most serious group of egg sac predators in the habitat, but the two adaptations, the hanging sacs and the impenetrable dirt layer, seem to have freed *B. lepida* more or less completely from this danger. Why the species at some localities produce attached sacs predominantly cannot be answered from the results presented here. One of the mechanisms protecting against ants, however, the dirt layer, functions also in the attached sacs.

Suspending the egg sac in the air by means of silk is a widespread phenomenon occurring in species of many families, either by a multitude of threads radiating from the egg sac to surrounding supports (e.g., Araneidae: Hieber 1992a, b; Theridiidae: Bristowe 1956; Uloboridae: Opell 1984), situated within a nest suspended in the web (Theridiidae: Nørgaard 1956; Bristowe 1956), or hanging from a single string or stalk (e.g., Agelenidae: Nielsen 1932; Mimetidae: Bristowe 1956; Theridiosomatidae: Hajer et al. 2009; Liocranidae: Holm 1940). Protection from predation, especially from ants, may be a universal function of these traits.

Use of debris or other materials from the surroundings to cover the egg sac can be seen as well in numerous spider families (Nielsen 1932). It is widespread in the family Agelenidae. Already Scheffer (1905) noted about *Agelenopsis naevia* (Walckenaer, 1842) "two silken blankets, with a layer of dirt or wood chippings between" forming the outer cover of the egg sac. *Tegenaria* spp. also may cover their sacs with debris (Nielsen 1932). Egg sacs placed in the open are often covered with debris, which may serve as visual camouflage in addition to physical protection against predators. Hidden sacs as well may be covered with debris (Nielsen 1932). In those cases, a physical protection function against predators may seem the best explanation, though chemical camouflage is also a possibility. In *Tegenaria*, the two traits (egg sacs hanging from a string and covered with debris) may even be combined (Nielsen 1932). A unique feature of the typical *B. lepida* hanging egg sac is that the dirt layer is completely covered by the outer silk layers (papery silk and outer flocculent silk layer) making the dirt layer undetectable from the outside.

ACKNOWLEDGMENTS

We thank the Blaustein Center for Scientific Cooperation for supporting the visit of ST to Sede Boqer. We are indebted to Dr. Gary Gibson, Canada, for identification of *Mercetina matritensis* and to two anonymous reviewers for valuable comments. This is publication no. 931 of the Mitrani Department of Desert Ecology.

LITERATURE CITED

Austin, A.D. 1985. The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian fauna. Journal of Natural History 19:359–376.

Barrantes, G., L. Sandoval, C. Sanchez-Quiros, P.-P. Bitton & S.M. Doucet. 2013. Variation and possible function of egg sac coloration in spiders. Journal of Arachnology 41:342–348.

Bristowe, W.S. 1956. The World of Spiders. Collins, London.

Christenson, T.E. & P.A. Wenzl. 1980. Egg-laying of the golden silk spider, *Nephila clavipes* L. (Araneae, Araneidae): functional analysis of the egg sac. Animal Behaviour 28:1110–1118.

Foelix, R. 2011. Biology of Spiders. 3rd ed., Oxford University Press, Oxford.

Hajer, J., J. Maly, L. Hruba & D. Rehakova. 2009. Egg sac silk of *Theridiosoma gemmosum* (Araneae: Theridiosomatidae). Journal of Morphology 270:1269–1283.

Hieber, C.S. 1985. The "insulation" layer in the cocoons of *Argiope aurantia* (Araneae: Araneidae). Journal of Thermal Biology 10:171–175.

Hieber, C.S. 1992a. The role of spider cocoons in controlling desiccation. Oecologia 89:442–448.

Hieber, C.S. 1992b. Spider cocoons and their suspension systems as barriers to generalist and specialist predators. Oecologia 91:530–535.

Holm, Å. 1940. Studien über die Entwicklung und Entwicklungsbiologie der Spinnen. Zoologiska Bidrag från Uppsala 19:1–214.

Lockley, T.C. & O.P. Young. 1993. Survivability of overwintering *Argiope aurantia* (Araneidae) egg cases, with an annotated list of associated arthropods. Journal of Arachnology 21:50–54.

Nielsen, E. 1932. The Biology of Spiders. Levin & Munksgaard, Copenhagen.

Nørgaard, E. 1956. Environment and behaviour of *Theridion saxatile*. Oikos 37:41–57.

Opell, B.D. 1984. Eggsac differences in the spider family Uloboridae (Arachnida: Araneae). Transactions of the American Microscopical Society 103:122–129.

Scheffer, T.H. 1905. The cocooning habits of spiders. Kansas University Science Bulletin 3:85–114.

Manuscript received 14 March 2017, revised 19 June 2017.