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Glue droplets in fossil spider webs

Липкие капли на ископаемых паутинах пауков

S. Zschokke

Department of Integrative Biology, Section of Conservation Biology (NLU), University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland. email: samuel.zschokke@alumni.ethz.ch

ABSTRACT. Amber is well known to conserve small insects and spiders, preserving the finest details of their morphology. However, spider webs in amber have so far largely been ignored, even though some webs in amber are superbly preserved; with even the smallest details being visible, like the glue droplets, which retain the prey in orb-webs and other araneoid webs. Here I present a brief introduction to the occurrence and function of glue droplets in Recent spider webs, followed by evidence of fossil glue droplets in Lebanese, Burmese, Baltic and Dominican ambers. A comparison of their states of preservation suggests that glue droplets in Lebanese amber have swollen up more than those in the other ambers. Finally, I discuss the evidence that these droplets are indeed fossilized glue droplets.

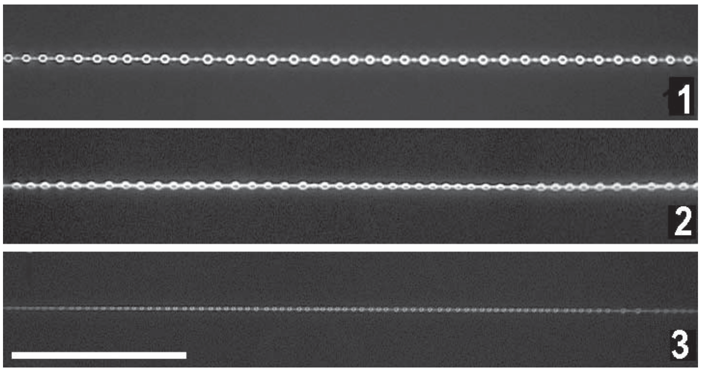
РЕЗЮМЕ. Хорошо известно, что янтарь содержит в себе мелких насекомых и пауков, сохраняя даже самые мелкие детали их морфологии. Однако, паутина пауков в янтаре до сих пор игнорировалась, несмотря на то, что некоторые образцы паутины великолепно сохранились, с видимыми мельчайшими деталями, такими как липкие капли, которые служат для удержания добычи в паутинах аранеоидных пауков. Здесь я даю краткое введение в наличие и функцию липких капель в паутинах современных сетей пауков, затем привожу доказательства существования липких капель в ливанском, бирманском, балтийском и доминиканском янтарях. Сравнение качества их сохранности позволяет заключить, что клейкие капли в ливанском янтаре более разбухшие, чем капли в других янтарях. В заключение, я обсуждаю доказательства того, что эти капли действительно фоссилизированные липкие капли.

KEY WORDS: Araneoidea, amber, glue droplet, spider silk, taphonomy, trace fossil, viscid silk, palaeontology.
КЛЮЧЕВЫЕ СЛОВА: Araneoidea, янтарь, липкие капли, шелк пауков, тафономия, следовое ископаемое, липкий шелк, палеонтология.

Introduction

Amber (polymerized fossil tree resin) has been known for a long time as a rich source of superbly preserved insects and other small organisms [e.g., Poinar & Poinar, 1999; Weitschat & Wichard, 2002]. Until recently, however, trace fossils in amber, i.e., fossils not of the organism itself, but of something the organism left behind, have been largely ignored. Among the most interesting trace fossils are spider webs, parts of which are occasionally superbly preserved [Poinar & Poinar, 1999], sometimes down to what appear to be glue droplets of the viscid silk [Bachofen-Echt, 1934; Zschokke, 2003].

Such droplets in fossil spider webs have been described several times. However, the origin of these droplets has been interpreted in different ways. The first description of spider webs in amber dates back to Menge [1856]. He



Figs 1–3. Glue droplets in Recent webs: 1 — *Araneus marmoreus* (Clerck, 1757); 2 — *Nephila* sp.; 3 — *Cyclosa walckenaeri* (O. Pickard-Cambridge, 1889). Scale: 500 µm.

Рис. 1–3. Липкие капли в современных паутинах: 1 — *Araneus marmoreus* (Clerck, 1757); 2 — *Nephila* sp.; 3 — *Cyclosa walckenaeri* (O. Pickard-Cambridge, 1889). Масштаб: 500 µm.

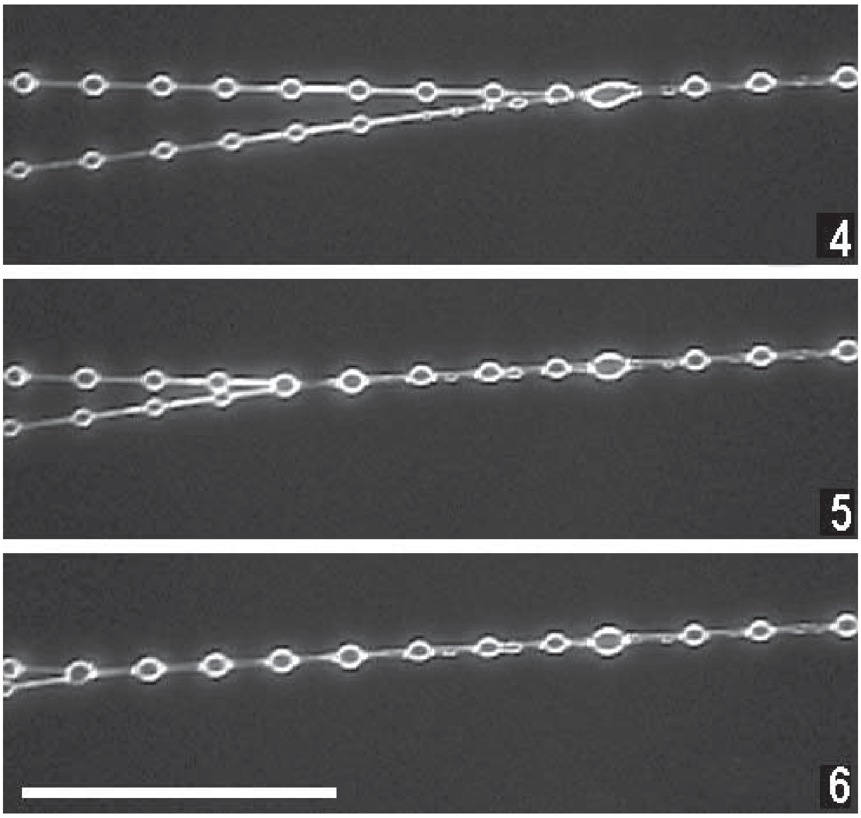
interpreted these droplets as resin droplets that had flowed down along the silk thread. Almost a century later, Bachofen-Echt [1934] described some spider webs with what he called "Klümpchen von Klebestoff" (small clumps of adhesive, i.e., glue droplets). Wunderlich [1986: 10, 227] figured two examples of spider silk with what he also considered to be glue droplets. Schlee [1990] on the other hand discussed the presence of small droplets along strands of spider silk, considering them to be resin droplets that had flowed along the silk and which had dried to a certain extent before they were embedded by a subsequent resin flow. Recently, Zschokke [2003] described a single thread with droplets, which he interpreted as glue droplets.

No previous study has systematically documented fossil droplets along strands of spider silk embedded in amber. The aim of this paper is to document droplets on spider silk in Lebanese, Burmese, Baltic and Dominican ambers, and to examine the evidence that suggests that these droplets are indeed fossilized glue droplets. In the first section, I present some information on Recent glue droplets. The second section of the paper is devoted to the description of fossil glue droplets from the various ambers and to the comparison of their state of preservation. In the final section, I present evidence supporting the hypothesis that these droplets are fossilized glue droplets. A review of fossil spider webs will be presented elsewhere.

Recent glue droplets

Glue droplets are typically found in webs built by araneoid spiders. Indeed, the aggregate silk glands, which produce the material for the gluey coating of sticky threads, have been suggested as the best single character defining the superfamily Araneoidea [Coddington, 1986]. The superfamily Araneoidea includes, among others, all ecribellate orb-weavers and the families Linyphiidae and Theridiidae. In ecribellate orb-webs, glue droplets are found exclusively on the sticky spiral [e.g., Zschokke, 2002], where they are used to capture and retain prey. In theridiid webs, glue droplets can be found either along the gumfooted lines or within the sheet, depending on the type of web [Benjamin & Zschokke, 2003]. In these webs, glue droplets are also used to capture and retain prey. In contrast, in linyphiid webs, where glue droplets are found in the sheet, glue droplets are used to cement the different layers of the sheet together [Benjamin *et al.*, 2002].

Glue droplets in orb-webs consist of a complex mixture of glycoproteins, covered by an aqueous solution of organic substances (mostly amino acids) and inorganic salts (KNO_3, KH_2PO_4) [for details see Vollrath *et al.*, 1990; Vollrath & Tillinghast, 1991], which makes them highly hygroscopic [Townley *et al.*, 1991]. In Recent orb-webs, the size of the glue droplets varies considerably between species, with diameters ranging from less than 10 µm to 200



Figs 4–6. Merging glue droplets of *Nephila* sp.: 4 — after 0 seconds; 5 — after 18 seconds; 6 — after 40 seconds. Scale: 500 μm.

Рис. 4–6. Слияние липких капель у *Nephila* sp.: 4 — через 0 секунд; 5 — через 18 секунд; 6 — через 40 секунд. Масштаб: 500 μm.

μm in undisturbed webs [Figs 1–3; Peters, 1987; Opell, 2002]. The largest glue droplets are probably found in the webs of some *Nephila* spp. (Tetragnathidae) [pers. obs.]. In linyphiid webs, droplet size also varies greatly, however, these droplets seem to be smaller than those in orb-webs [2–10 μm diameter; Peters & Kovoor, 1991; Benjamin *et al*., 2002]. For some species, it is characteristic for the glue droplets along a thread to alternate in size between large and small ones [Fig. 1; Vollrath, 1992]. When an orb-web is damaged and threads with glue droplets have come into contact with each other, these threads will tend to slowly merge into one thread, which then bears droplets of increased size and a less regular arrangement (Figs 4–6). This merging is most likely caused by the surface tension of the droplets.

Fossil glue droplets

Here, I present data of fossil spider silk with what I consider to be fossil glue droplets (see below) from four different types of amber, ordered by age. The uniformity of the size of the glue droplets within each specimen was assessed by calculating their coefficient of variation (C.V.). The following abbreviations for collections are used in the text: JW = Jörg Wunderlich's collection (Hirschberg-Leutershausen, Germany); MEW = Museum of the Earth, Warsaw, Poland (Dr. J. Kupryjanowicz); SMNS = Staatliches Museum für Naturkunde, Stuttgart, Germany (Dr. G. Bechly); SZ = author's personal collection. Absolute ages are based on the IUGS [2000].

Lebanese amber

Lebanese amber collected near Jezzine is considered to be the oldest amber with animal inclusions and is dated to the late Valanginian to

Table.
List of amber specimens containing spider silk with glue droplets. Median droplet size and range (in parentheses) is given, as well as the Coefficient of Variation (C.V.) and the number of droplets.

Таблица.
Список образцов янтаря, содержащих паучью паутину с липкими каплями. Даны средний размер капли и вариации (в скобках), а также Коэффициент Вариации (C.V.) и количество капель.

Amber type	Age (Mya)	Botanical source	Specimen	Droplet size (µm)	C.V.	# droplets
Lebanese	130–135	*Agathis levantensis* (Coniferales: Araucariaceae)	SMNS: 19/2	12 (7–85)	1.02	38
Burmese	95–105	*Metasequoia* sp. (Coniferales: Cupressaceae)	SZ: M 5	40 (13–275)	0.84	65
			SZ: M 15	41 (17–116)	0.61	22
Baltic	35–50	*Pinites succinifer* or *Pseudolarix* sp. (Coniferales: Pinaceae)	SZ: B 14	119 (42–385)	0.60	31
			SZ: B 18	19 (8–55)	0.57	42
			MEW: 20493TG	19 (9–113)	0.77	109
Dominican	15–20	*Hymenaea protera* (Fabales: Fabaceae)	SZ: D 38	25 (8–456)	1.26	102
			SZ: D 40	129 (29–1250)	1.16	78
			SZ: D 41	76 (25–778)	1.15	43
			SZ: D 46	51 (17–574)	1.30	45
			SZ: D 47	30 (12–812)	1.62	28

Hauterivian stage of the Lower Cretaceous (*c.* 130–135 Mya = Million years ago) [Schlee & Dietrich, 1970]. The botanical origin of Lebanese amber is thought to be the Kauri pine (*Agathis levantensis*, Coniferales: Araucariaceae) [Lambert *et al*., 1996; Poinar & Milki, 2001].

A single specimen with a short piece of spider thread containing glue droplets has recently been described from Lebanese amber (SMNS: 19/2) [Zschokke, 2003], representing the oldest direct evidence for viscid silk and for spider webs. In this specimen, 38 distinct glue droplets can be distinguished, mostly with diameters between seven and 29 µm. Five droplets are considerably larger, with diameters up to 85 µm (Table).

Burmese amber

Burmese amber is now dated to the Late Albian or the Cenomanian stage of the Middle Cretaceous (*c*. 95–105 Mya) [Zherikhin & Ross, 2000; Grimaldi *et al*., 2002; Cruickshank & Ko, 2003]. The botanical origin of Burmese amber is thought to be *Metasequoia* sp. (Coniferales: Cupressaceae) [Grimaldi *et al*., 2002].

For this study, two Burmese amber specimens containing silk with droplets were available. In one specimen (Fig. 7), 65 droplets are distinguishable, mostly with diameters between 13 and 70 µm. Nine droplets are larger with diameters up to 275 µm. In the other specimen, only 22 droplets are visible, with diameters ranging from 17 to 116 µm.

Baltic amber

Baltic amber constitutes most of the amber known today and is generally considered to have been formed in the Eocene, 35–50 Mya. The botanical origin of Baltic amber is thought to be *Pinites succinifer* or *Pseudolarix* sp. (Coniferales: Pinaceae) [Grimaldi, 1996].

Three specimens were available for this study. In two specimens, the droplets are rather small (Fig. 8, median = 19 µm), whereas one specimen features larger droplets (Fig. 9, median = 119 µm). Nevertheless, the size of the droplets within each specimen is fairly uniform, with coefficients of variance smaller than 0.8 (Table).

Dominican amber

Dominican amber generally contains the best preserved amber fossils. It is dated at late Early Miocene to early Middle Miocene, 15–20 Mya [Iturralde-Vinent & MacPhee, 1996]. The botanical origin of Dominican amber is thought to be the algarroba tree (*Hymenaea protera*, Fabales: Fabaceae) [Poinar & Poinar, 1999].

For this study, five specimens were available. It is striking, how large some of the droplets in each of these specimens are (Fig. 10, Table). Each of the five specimens contains some droplets with diameters larger than 400

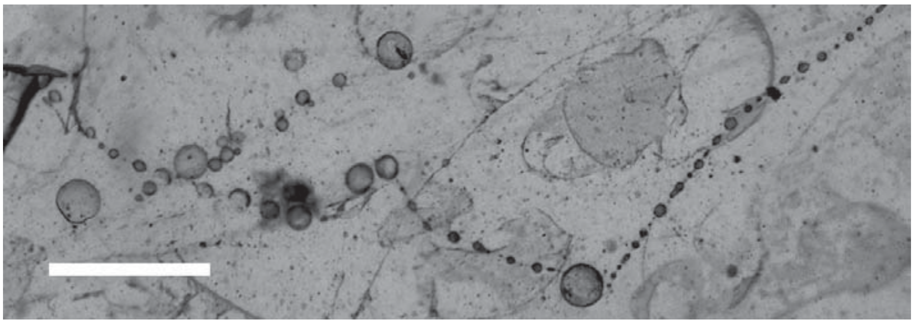
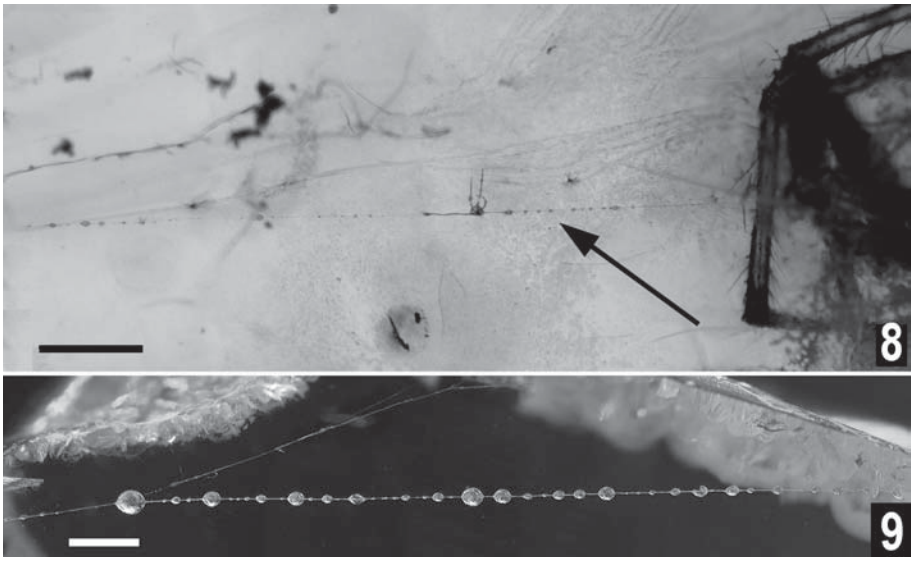


Fig. 7. Silk strands with glue droplets in Burmese amber (SZ: M 5). Scale: 1 mm.

Рис. 7. Нити паутины с липкими каплями в бирманском янтаре (SZ: M 5). Масштаб: 1 мм.



Figs 8–9. Silk strands in Baltic amber: 8 — silk with glue droplets (arrow) near an araneoid spider (visible are just some legs of a Synotaxidae, ?*Acrometa* sp., det. JW; SZ: B 18); 9 — Silk with and without glue droplets (SZ: B 14). Scale: 1 mm.

Рис. 8–9. Нити паутины в балтийском янтаре: 8 — паутина с липкими каплями (помечена стрелкой) возле аранеоидного паука (видны только несколько ног Synotaxidae, ?*Acrometa* sp., опред. JW; SZ: B 18); 9 — паутина с липкими каплями и без них в балтийском янтаре (SZ: B 14). Масштаб: 1 мм.

Fig. 10. Silk with glue droplets in Dominican amber, showing glue droplets which have swollen up, probably through the uptake of water (SZ: D 46). Scale: 1 mm.

Рис. 10. Паутина с липкими каплями в доминиканском янтаре, на которой липкие капли вздулись, видимо, из-за того, что впитали воду. (SZ: D 46). Масштаб: 1 мм.

µm, one even contains two droplets with a diameter larger than 1 mm. At the same time, all specimens also contain a large number of small droplets, resulting in a high variability of droplet sizes (C.V. > 1.1 for all specimens).

Preservation of droplets

The preservation of the droplets differs between the different types of amber. Whereas droplets more or less retained a uniform size within each specimen in Baltic and Burmese amber, the droplets in Dominican amber show a higher size variability than those in the other types of ambers (Table; ANOVA, $F_{3,7} = 11.58$, $P = 0.004$). I have observed a similar pattern in a few more specimens of Baltic and Dominican ambers. However, I could not include them in this analysis due to lack of measurements. If we assume that the droplets were reasonably uniform in size before the thread was engulfed in resin, the following questions remain to be answered: (a) why did some glue droplets increase in size? and (b) why is the size variability of the droplets in Dominican amber so much higher than in the other ambers analyzed?

Since glue droplets are highly hygroscopic [Townley *et al.*, 1991], any water in close proximity to them will be taken up, leading to the observed increase in size. An uneven size increase of the droplets can thus be explained by an uneven availability of water to the droplets, either due to a variable water content of the resin or a variable water permeability of the resin. We can only speculate about the reason why the size variability of the droplets in Dominican amber is higher than in other amber types. One reason could be the botanical origin of the resin. Whereas the older ambers stem from coniferous resin, the origin of Dominican amber is a deciduous tree. It is thus possible that the different preservation states of glue droplets in Dominican amber are caused by a difference in resin composition between the two plant groups.

Origin of the droplets in fossil spider webs

As outlined in the introduction, there are two competing hypotheses concerning the origin of the droplets in spider webs in amber: the droplets were either droplets of resin or glue droplets produced by spiders. The idea that glue droplets have been preserved in amber may initially seem surprising, since glue droplets and resin are both liquid. However, a closer analysis reveals that the glue droplets hypothesis is not as unlikely as it may at first appear. Firstly, the shape, arrangement and size of the fossil glue droplets in most cases closely matches (except some distended droplets in Dominican amber) that of glue droplets in Recent spider webs, especially if we consider that some of the fossil spider threads are likely to have merged before or during engulfment in resin and the droplets thus increased in size and have become less regular (Figs 4–6).

Judging from the superb preservation of glue droplets in several specimens (e.g., Fig. 9), it is quite likely that the resin that covered these threads had a low viscosity, otherwise the droplets would probably have been destroyed by the force of the resin flow. It can be easily understood why the glue droplets did not mix with the resin. Glue droplets consist of proteins and water [Vollrath *et al.*, 1990] and the resin, consisting mainly of terpenoids [Langenheim, 1990], is highly hydrophobic. The two substances repel each other and therefore do not mix.

A small experiment shows that glue droplets can indeed remain intact under such conditions: when a fragment of a Recent orb-web was immersed in turpentine oil (terpenoids extracted from pine resin), the glue droplets stayed perfectly intact (Fig. 12). Removing the silk with the glue droplets from the oil affected them only minimally (some droplets merged). When a similar experiment was repeated with water instead of turpentine oil, the glue droplets largely dissolved in the water and no remains of them were obvious when the thread was taken out of the water. Similarly, it is also well known that fossil water droplets have remained intact in amber [Buchberger *et al.*, 1997]. However, in contrast to these water droplets, the glue droplets have become solid like the surrounding amber matrix.

The hypothesis that these droplets are fossil glue droplets is further supported by the regularity and symmetry of the droplets in many

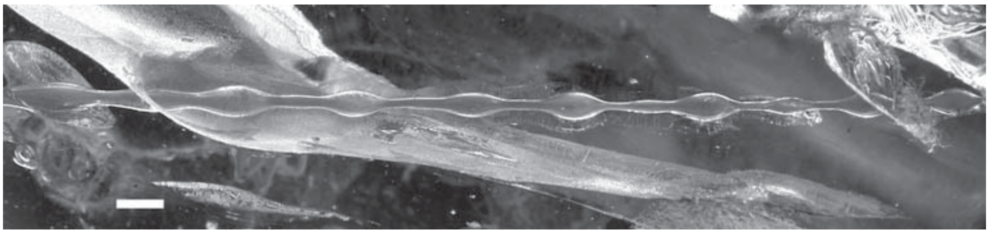


Fig. 11. Thin linear object (?spider silk) with resin droplets in Baltic amber (JW: F919). Scale: 1 mm.

Рис. 11. Тонкий линейный объект (?паутина паука) с каплями смолы в балтийском янтаре (JW: F919). Масштаб: 1 мм.

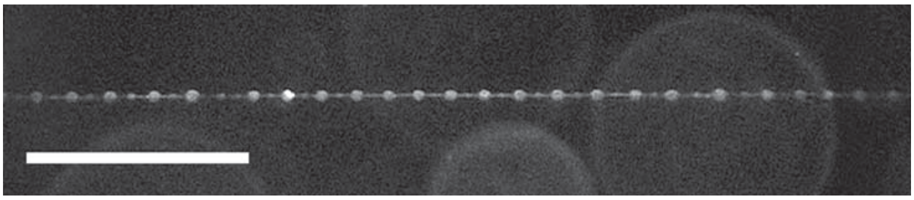


Fig. 12. Recent silk with glue droplets immersed in turpentine oil. Scale: 500 μm.

Рис. 12. Современная паутина с липкими каплями, помещенная в скипидарное масло. Масштаб: 500 μm.

pieces of amber, the fact that some threads in one piece have droplets, and other, neighbouring threads do not have them (Fig. 9), and the fact that we cannot find other linear objects with such small resin droplets. Droplets that can be identified as resin droplets do occur, but they are much larger and have a different shape compared to the droplets in spider webs (Fig. 11). Finally, there are amber specimens where a thread with glue droplets is embedded near an araneoid spider, like the one shown in Fig. 8. All these considerations strongly support the hypothesis that the droplets along the spider threads are really fossilized glue droplets.

Conclusions

The degree of swelling of the droplets in Dominican amber is greater than in the other ambers studied (Lebanese, Burmese and Baltic). I suggest that this difference is related to the botanical origin of the amber forming resin.

From the available evidence I conclude that the droplets found in fossil spider webs in amber are indeed fossilized glue droplets. Thus, it can be inferred that the use of silk with glue droplets dates back to at least the lower Cretaceous, confirming the conclusions drawn from the fossil araneoid spiders from the same period [Selden, 1989; Penney & Selden, 2002].

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