

Fossil evidence for the origin of spider spinnerets

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Silk production from opisthosomal glands is a defining characteristic of spiders (Araneae). Silk emerges from spigots (modified setae) borne on spinnerets (modified appendages). Spigots from *Attercopus fimbriunguis* (Shear, Selden & Rolfe, 1987¹), from Middle Devonian (386 Ma) strata of Gilboa, New York were described in 1989² as evidence for the oldest spider and the first use of silk by animals. Slightly younger (374 Ma) material from South Mountain, New York, conspecific with *A. fimbriunguis*, includes spigots and other evidence which elucidate the evolution of early Araneae and the origin of spider silk. No known *Attercopus* spigots, including the original specimen², occur on true spinnerets but are arranged along the edges of plates. Spinnerets originated from biramous appendages of opisthosomal somites 4 and 5; while present in *Limulus*, no other arachnids have opisthosomal appendage homologues on these segments. The spigot arrangement in *Attercopus* shows a primitive state prior to the re-expression of the dormant genetic mechanism which gave rise to spinnerets in later spiders. The inability of *Attercopus* precisely to control silk weaving suggests its original use as a wrapping, lining or homing material.

The oldest silk-producing spigots are known from the Middle Devonian of Gilboa, New York². This specimen, slide 334.1b.AR34 (Fig. 1a), was described as a nearly complete, fusiform spinneret, consisting of a single article, bearing about 20 spigots arrayed along the presumed medial surface but more clustered distally. On the basis of the single, simple spigot type and the lack of tartipores (vestigial spigots from earlier instars), the fossil spinneret was compared most closely with those of the primitive spider suborder Mesothelae, and was presumed to be a posterior median spinneret. The distinctiveness of the cuticle enabled us to associate the spinneret with remains previously referred tentatively to a trigonotarbid genus¹. Restudy of this material resulted in a fuller description of the animal as the oldest known spider, *Attercopus fimbriunguis*³. The appendicular morphology of *Attercopus*, but little of the body, is now known in great detail.

Collections made in 1993 and 1996 at the South Mountain locality, Schoharie County, New York (74°16'30"E/42°23'55"N), in Middle Devonian strata (lower Frasnian, lowermost Onteora Formation, 374 Ma⁵), yielded new material which is indistinguishable from *Attercopus fimbriunguis* from Gilboa, and thus presumed to be conspecific. The new material includes three pairs of chelicerae (thus establishing the presence of at least three individuals), numerous podomeres including a palpal femur showing the distinctive patch of spinules on the infero-anterior surface (Fig. 1b), and two slides with specimens showing spigots. The last are numbered sequentially (SM 1.11.3 and SM 1.11.4), which means they were extracted from the the same acid-macerate residue and slide-mounted one after another, and so could be parts of the same animal. SM 1.11.3 (Fig. 1e) consists of a subrectangular mass of overlapping layers of cuticle with about 33 spigots arrayed in an approximate double row along one long edge and an area of unsculptured cuticle along the opposite edge. The folds have their long axes parallel to the shorter edges. These features, together with the setal arrangement, suggests to us that the preferred orientation is:

unsculptured cuticle anterior, spigots posterior, shorter edges lateral. Seven macrosetae and/or their sockets are present on SM 1.11.3. One postero-lateral corner is missing; spigots are most numerous at the opposite postero-lateral corner. Because of the presence of spigots, we interpret SM 1.11.3 as part of the opisthosoma. Living and fossil mesotheles have macrosetae at the rear of each large tergite⁶, and other spiders which lack tergites commonly bear large setae on the abdomen which reflect original segmentation; thus the macrosetae on *Attercopus* SM 1.11.3 could also reflect at least four sclerotized plates and the transverse lines could represent plate boundaries (NB both dorsal and ventral surfaces are present).

SM 1.11.4 (Fig. 1c,d) is a smaller piece of cuticle than SM 1.11.3. The distribution of setae and spigots enables orientation of the piece. At one lateral side is an even fold that conforms to the curved outline of the postero-lateral margin; this is interpreted as a double fold along the margin of the plate. It is folded at the lateral side and bears about 15 spigots in an approximate double row along the posterior edge; the anterior and opposite lateral edges are torn. If SM 1.11.4 were once joined to SM 1.11.3, then it is likely that it is the missing postero-lateral corner of SM 1.11.3, with its postero-lateral concentration of spigots. Of especial interest on SM 1.11.4 is the long, winding, filament emerging from the distal end of one spigot (Fig. 1d). Detailed study shows that this is a single strand which is inseparable microscopically from the tip of the spigot, thus leading us to hypothesize that this is a strand of silk. No other silk strands have been seen in *Attercopus* material, but silk from modern spiders is identical in size and appearance under the light microscope.

From our re-evaluation of 334.1b.AR34 we conclude that the original description is essentially correct, but note that the specimen consists of a sheet of cuticle folded over twice; thus the resemblance of the piece of cuticle bearing spigots to a 'semifusiform'

spinneret³ is fortuitous. In summary, the specimens of *Attercopus* bearing spigots are plate-like in morphology, with two rows of spigots along the presumed posterior edge. The spigots are not borne on appendage-like spinnerets.

Spider spinnerets are homologues of biramous opisthosomal appendages, still present in the primitive chelicerate *Limulus*, as demonstrated by expression of the developmental genes *pdm/nubbin* and *apterous* in embryos of spiders and *Limulus*⁷. In *Limulus* these appendages consist of a segmented median branch and a lateral branch with a plate covering lamellate gills. In spider embryos, *distalless* gene expression shows four pairs of spinnerets (anterior median and lateral, and posterior median and lateral pairs) represented by two pairs of appendage buds on opisthosomal somites 4 and 5⁸. The appendage buds each later divide in two to produce potentially four pairs of spinnerets, although in nearly all spiders some of these buds do not develop into functional post-embryonic spinnerets. The full complement of eight spinnerets is today seen only in the primitive mesotheles *Liphistius* and *Heptathela* (even in these animals the anterior median pair bears no silk-producing spigots)⁹. Other homologues of opisthosomal appendages in spiders are the book-lung opercula (2 pairs in mesotheles and mygalomorphs, on somites 2 and 3) and tracheae derived from appendage apodemes in araneomorph spiders on somite 3. The book-lung covers and pectines of scorpions and Blattfüsse of eurypterids are similarly derived from opisthosomal appendages. In other arachnids, homologues of opisthosomal appendages can be seen in the gonopods and book-lung opercula of tetrapulmonates, and possibly other organs in diverse groups¹⁰, but only spiders show expression of appendage homologues on somites 4 and 5. Silk glands also occur in many adult male spiders along the anterior edge of the epigastric furrow (somite 2). These are termed epiandrous or epigastric glands¹¹, and open through simple spigots (fusules). Epigastric fustules are

simple spigots which, because of their medial position in relation to the more lateral book-lung opercula, could be serial homologues of the median spinnerets of somites 4 and 5.

The advantage of spigots on spinnerets is obvious: silk production can be controlled to produce complex linear structures, rather than just sheet-like masses of threads. Our interpretation of spigot location in *Attercopus* suggests that the original use of silk in proto-spiders was to produce such sheets, perhaps used as burrow linings, or to cover egg masses, or as trails that would allow hunting animals to return to the safety of a retreat¹².

Loss and reappearance of wings in stick insects suggests that genes for appendage development can be suppressed, perhaps by a single disabling mutation, and later reactivated, again perhaps by a reversal of the original mutation or an offsetting mutation that restores gene function¹³. Once these genes were reactivated in the ancestors of spiders, it would be a clear advantage to have the spigots on them as this would confer significantly more control over the use and distribution of silk, as seen in the orb-weaving Orbiculariae of today in the construction of their architecturally precise webs.

While mesothele spiders and a few mygalomorphs have abdominal tergites that can be attributed to the original segmentation of the abdomen, no spiders living or fossil have ventral abdominal plates. However, these plates are present in all other arachnid orders, including the Pedipalpi (orders Amblypygi, Uropygi and Schizomida). The origin of these plates is not well understood and the patterns of expression of *hox* genes has not been studied except in spiders and some mites (in the latter with focus on head segmentation, not expression of appendage-determining genes¹⁴). It has been suggested on the basis of palaeontological and developmental evidence¹⁵, at least for scorpions, that these plates are not true sternites but are in fact the fused remnants of paired abdominal appendages, as

indeed seems to be the case for the epigastric plate and book lung covers of spiders. In mesotheles the first two pairs of book-lung covers are part of continuous sclerotization across the abdomen, with distinct posterior margins.

It seems highly unlikely that the spigot-bearing plates in *Attercopus* are tergites, and much more probable that they represent ventral plates, since in spiders the spinnerets are invariably ventral. If the ventral plates are appendage-derived, the reactivation of genes (such as *distalless*) which would extend these plates once more into segmented appendages would carry along with them the spigots observed in *Attercopus* to be distributed along the posterior margins of those plates. We suggest that developmental genetic studies to determine the homologies of the ventral plates in the pedipalp orders could provide evidence to resolve this question. Further evidence that silk spigots are associated with appendages comes from the recent finding that at least one species of mygalomorph spider has silk spigots on the ventral surfaces of its leg tarsi¹⁶. These spigots produce threads that help the spider cling to smooth surfaces.

In *Permarachne*, from the Permian of Russia, a series of six abdominal plates are clearly seen¹⁷ (Fig. 2a,b). In the original description these were interpreted as tergites (as seen in mesotheles) even though all other visible structures in the fossil are ventral, a fact originally accounted for by assuming that the specimen represented a moult from which the carapace had been displaced, thus revealing ventral structures in the prosoma. However, these structures are in ventral, not dorsal, view. It now seems more parsimonious to interpret the series of plates as ventral plates, conforming to the ventral view of the rest of the fossil. Thus there is a real probability that both *Attercopus* and *Permarachne* bore a series of ventral plates.

In summary, we propose the following scenario for the origin of spinnerets, based on our restudy of *Attercopus* and *Permarachne*. The ancestors of spiders had ventral opisthosomal plates, possibly derived from appendages, along the edges of which were arrayed silk-producing spigots. The reactivation of hox genes controlling appendage development (possibly *distalless* or a similar gene) caused the redevelopment of appendages on at least two opisthosomal segments, and the spigots were carried along onto these nascent spinnerets. The greater control thus achieved over silk-weaving triggered the enormous radiation of spider diversity. It has not escaped us that under this scenario, *Attercopus* and *Permarachne* may no longer be considered spiders, and we will describe in another place an ordinal-level plesion to receive them.

Methods

Attercopus fimbriunguis specimens were recovered from the rock matrix by digestion in concentrated hydrofluoric acid followed by washing in dilute hydrochloric acid and mounted on plain microscope slides in Clearcol mountant. The specimens were studied using a Leica DM2500 M microscope and photographed with a Leica DFC420 digital camera attachment. Images of the new *Attercopus* specimens were captured using Leica FireCam software on an Apple MacBook Pro computer and manipulated using Adobe Photoshop CS3 software. Drawings were made using a drawing tube attached to the microscope and also by tracing photographic images in Adobe Illustrator CS3. All specimens are deposited in the Department of Invertebrates, American Museum of Natural History, New York.

The holotype and only known specimen of *Permarachne novokshonovi*, PIN 4909/12, part and counterpart, comes from the Koshelevka Formation, Kungurian Stage, Cisuralian Series (Permian), at the Krutaya Katushka outcrop, left bank of the Barda River, upstream

of Matveyevka, Russia, and is deposited in the Palaeontological Institute of the Russian Academy of Sciences, Moscow¹⁷. The specimen was studied, under ethanol to enhance contrast, using a Wild M7S stereomicroscope, drawn using a drawing tube, and photographed with a Nikon D1X digital camera attached to the microscope.

<received> Style tag for received and accepted dates (omit if these are unknown).

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Figure 1. *Attercopus fimbriunguis*, Devonian of New York (localities: G = Gilboa, SM = South Mountain), macerated from matrix with HF and slide-mounted. a, first-described ‘spinneret’, G 334.1b.34; darkness of cuticle reflects number of layers, so this fragment is folded over twice. b, distinctive palpal femur, SM 1.11.12. c, piece of cuticle from corner of opisthosomal ventral plate showing setae, spigots and possible silk strand, SM 1.11.4. d, close-up of e showing possible silk strand emerging from spigot shaft, SM 1.11.4. e, part of opisthosoma with double row of spigots, SM 1.11.3. Scale bars, 0.5 mm.

Figure 2. *Permarachne novokshonovi*, Permian of Russia, PIN 4909/12. a, holotype part in rock matrix. b, Explanatory drawing of a. ch, chelicera; cx, coxa; fe, femur; mt, metatarsus; pa, patella; pl, ventral plate; st, sternum; ta, tarsus; ti, tibia. Scale bar, 1 mm.



