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SHORT COMMUNICATION

Silk gland morphology of the net casting spider *Deinopis spinosa* (Araneae: Deinopidae)

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Abstract. Net-casting spiders (Deinopidae) are cribellate spiders that spin a rectangular, sticky net that is held stretched between the claws of their first two pairs of legs. Deinopids produce eight distinct silk types, but knowledge of the silk-producing morphologies is mostly limited to the spigots associated with different fibers. As there have been no studies of deinopid silk gland structure, we dissected all the silk glands from *Deinopis spinosa* Marx, 1889 and document their number and morphology. We found silk gland position and morphology consistent with the type and number of silk spigots described for Deinopidae. Notably, for the first time, we describe the silk glands associated with cribellate silk: paracribellate, pseudoflagelliform, and cribellar silk glands. Our findings support the homology of pseudoflagelliform glands with araneoid flagelliform glands and will have importance for informing our understanding of spider web evolution.

Keywords: Deinopid, cribellate silk, silk glands, spigot, spinneret
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Net-casting spiders (Deinopidae) are skilled nocturnal hunters. These cribellate spiders spin an unusual web: a rectangular, sticky net that the spider holds stretched between the claws of its first two pairs of legs. Suspended in a frame web of non-sticky silk, the spider hovers motionless at night, then when prey is detected, it rapidly expands its net and flings it over prey moving on the substrate beneath them or flying above them (Coddington & Sobrevila 1987). Studies on *Deinopis spinosa* Marx, 1889 (Marx 1889) have shown that this unique foraging strategy involves multiple specializations, hyper-sensitive night-vision to capture prey off the ground (Stafstrom & Hebets 2016), hearing to catch prey out of the air (Stafstrom et al. 2020), and extremely extensible silk (Blackledge & Hayashi 2006).

Deinopids use a dry, Velcro®-like adhesive in their prey-capture web, composed of cribellar silk surrounding a pair of supporting fibers, a pair of undulating fibers, and a mat of connecting fibers. Cribellar silk is produced from a specialized plate called the cribellum. The cribellum is densely covered with thousands of miniature spigots from which nanofibrils emerge and then are teased into highly-coiled, cloudy masses of fibers. The supporting fibers are produced in the pseudoflagelliform glands, the undulating fibers in the minor ampullate silk glands, and the connecting fibers in the paracribellate silk glands (Peters & Kovoor 1980; Peters 1984, 1992). While the external spinning apparatus (spinnerets and their spigots) has been characterized in a few works (Coddington 1989; Peters 1992; Griswold et al. 1998, 2005; Murphy & Roberts 2015), to our knowledge, there are no previous morphological studies of the silk glands associated with deinopid spigots.

Here, we document all silk gland types present in the net-casting spider *D. spinosa* and describe their position, size, and morphology, drawing comparisons with current knowledge about their spigots, as well as silk gland morphology outside of deinopids. Our findings are consistent with the number and variety of spigot morphologies and for the first time for any spider, we describe the morphology of the silk glands used in the cribellate capture silk.

Mature female *D. spinosa* were collected in Florida (2021 and 2022). All spiders were anesthetized with CO_2 and dissected under a stereomicroscope with forceps in saline sodium citrate buffer. Images of all tissues were collected from two individual females using a ZEISS Axiocam 105 Color Microscopy Camera mounted on a ZEISS 435063-9010-100 Stemi 305 Stereo Microscope with the ZEN Blue software. We identified eight types of silk glands: aciniform, cribellar, major ampullate, minor ampullate, paracribellate, pseudoflagelliform, piriform, and tubuliform (Fig. 1), which correspond to the eight types of silk spigots described for deinopid spiders (Fig. 2) (Coddington 1989; Peters 1992; Griswold et al. 1998; Murphy & Roberts 2015). Each gland type was confirmed by tracing its duct to the spinneret on which it terminated. Penultimate and mature males were dissected using the same procedure. While no images were taken for males, we noted that mature males do not have tubuliform, pseudoflagelliform, paracribellate, or cribellar silk glands. An additional mature female was anesthetized with CO_2 and the abdomen was severed at ~30% anterior of the spinnerets. The spinnerets were prepared following Townley & Harms (2017). In short, spinnerets were submerged in 2X Novex Tris-Glycine SDS Running Buffer (ThermoFisher USA LC2675) for 3 days, then dehydrated in an ethanol series: 30%, 50%, 70%, 85%, 95%, and then twice in 100% (24 hour each). Spinnerets were critical-point dried using a Tousimis Samdri Critical Point Drier, mounted on SEM stubs, sputter-coated with a Denton Vacuum Desk IV, and examined in a JEOL 6390 scanning electron microscope.

The major ampullate and minor ampullate glands were astonishing in number and shape. There were ten pairs of major ampullate glands (Fig. 1A), corresponding to the ten major ampullate spigots on each anterior lateral spinneret (ALS; Fig. 2A, B) (Coddington 1989; Peters 1992). By contrast, most mature araneoids have only one pair of major ampullate glands spigots (Coddington 1989). Many non-araneoid spiders, such as eresids, nicodamids, desids, amaurobiids, agelenids, and lycosoids, have been described as having more than one pair of major ampullate silk spigots as mature individuals

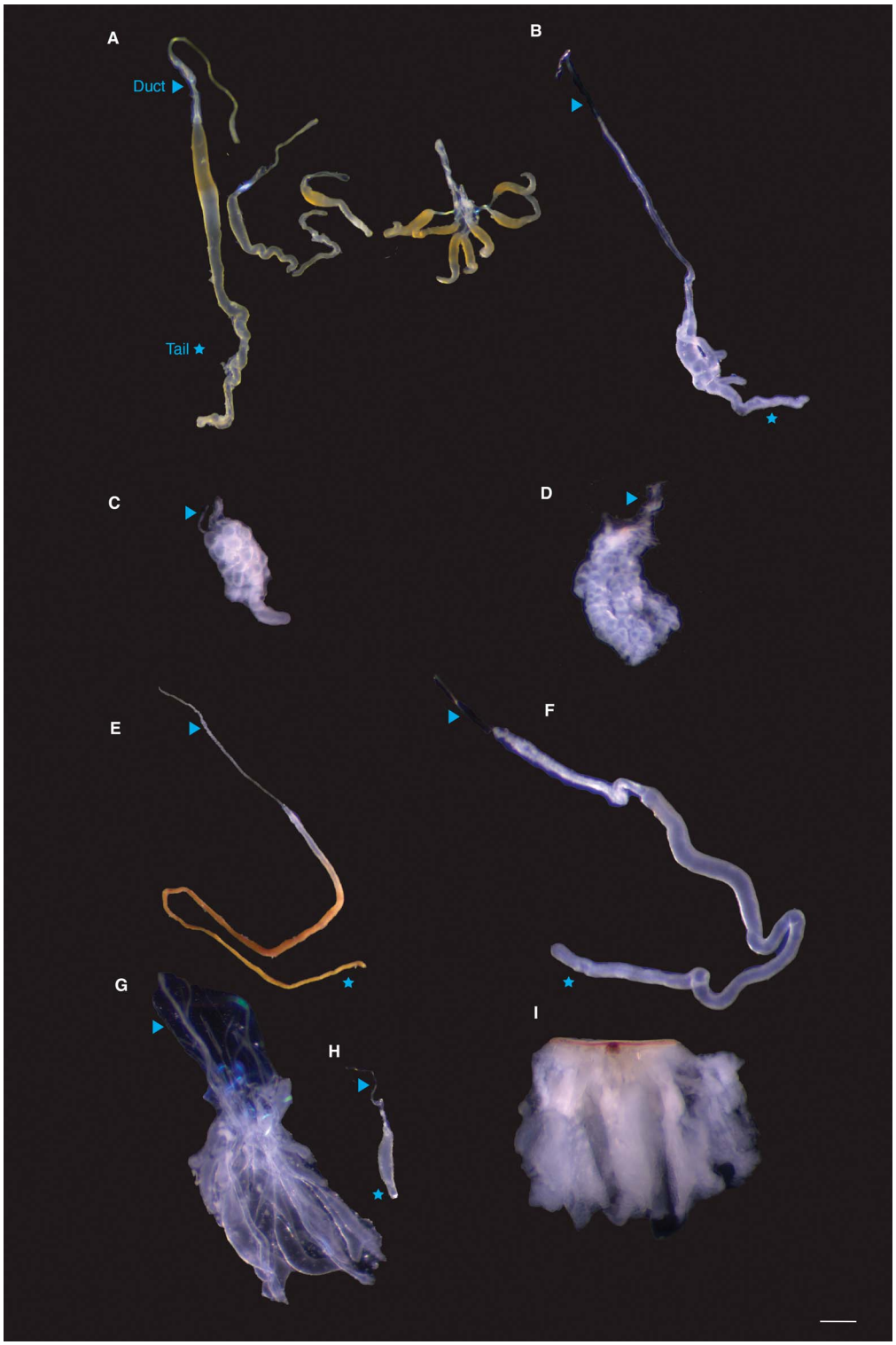

Figure 1.—Silk glands of the net-casting spider *Deinopis spinosa*. Silk glands are oriented with the ducts (arrow) up and tails (star) at the bottom. A. Major ampullates, B. Minor ampullate, C. Aciniform cluster, D. Piriform cluster, E. Tubuliform single, F. Pseudoflagelliform, G. Paracribellate cluster, H. Paracribellate single, and I. Cribellar cluster with cribellar cuticle (top). Major ampullate, minor ampullate, aciniform cluster, piriform cluster, pseudoflagelliform, and paracribellate are paired inside the spider and only one side is depicted. Raw images were placed on black background using Photoshop without further modification. Scale bar = 500 μm.

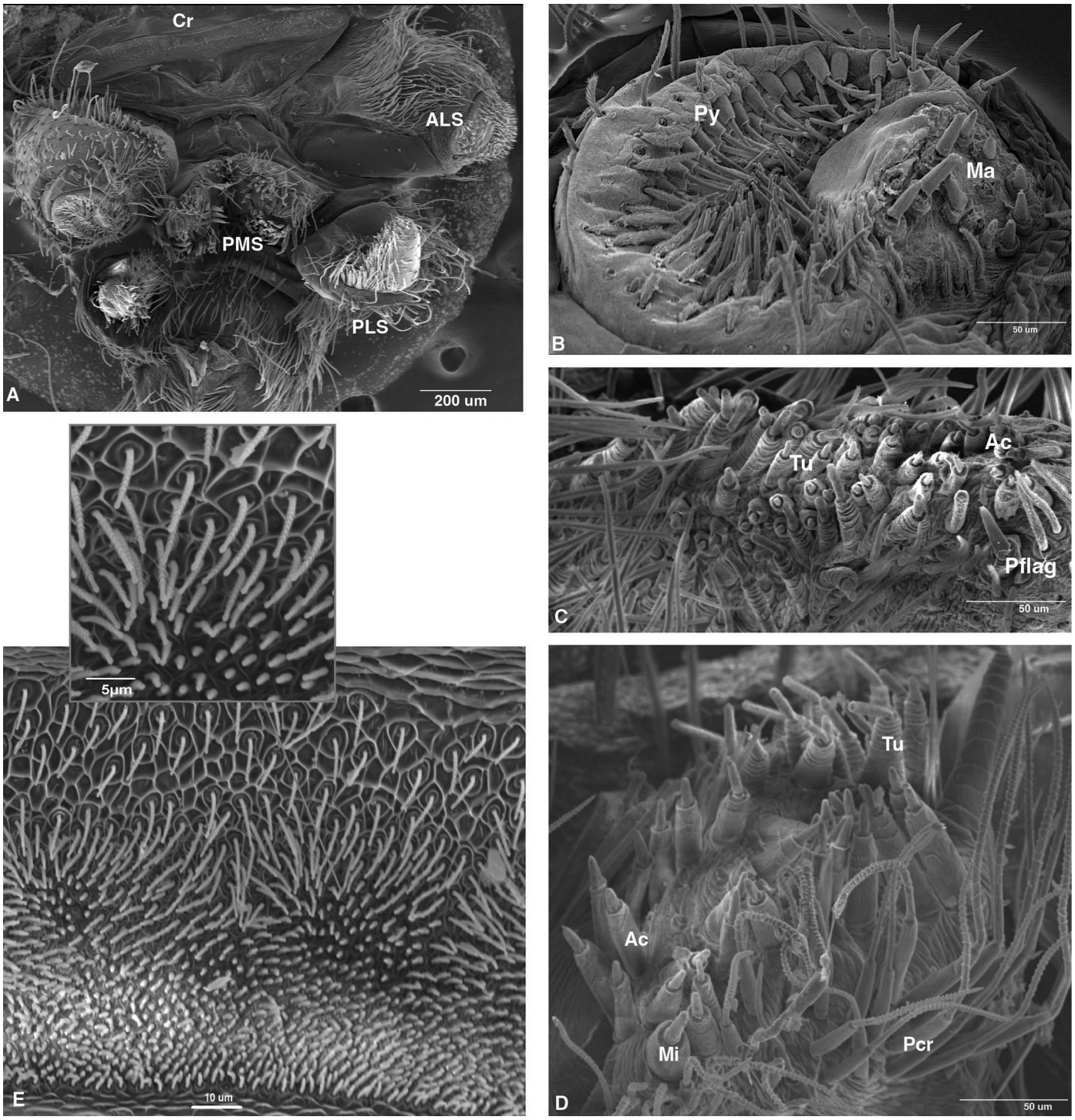

Figure 2.—Cribellum and spinnerets of adult female *Deinopis spinosa*. A. Overview of spinnerets B. Anterior Lateral Spinneret (ALS), C. Posterior Lateral Spinneret (PLS), D. Posterior Median Spinneret (PMS) E. Cribellum, with close-up inset on upper left. Abbreviations as follows: Cr cribellum and spigot types, Py: piriform, Ma: major ampullate, Tu: tubuliform, Ac: aciniform, Pflag: pseudoflagelliform, Mi: minor ampullate, Pcr: paracribellate.

(Griswold et al. 1998, 2005; Řezáč et al. 2017). Similarly, most juvenile spiders have multiple major ampullate spigots which have been proposed to be used alternatively during molting. That is, one spigot functions in even-numbered instars and the other in odd-numbered instars (Townley et al. 1993). The silk glands associated with these spigots also undergo changes during ecdysis: larger ampullate glands (usually those that stay through maturity) become non-functional pre-ecdysis while the smaller ampullate silk glands become functional, thus allowing for continuous use of ampullate silk.

We also found the elongated *D. spinosa* major ampullate glands to vary in size within an individual, a phenomenon rare among araneid and theridiid spiders (Coddington 1989; Chaw & Hayashi 2018; Berger

et al. 2021). However, such variation has been described for some agelenid and cribellate amaurobiid species (Řezáč et al. 2017). In the case of agelenids, Řezáč et al. (2017) showed that they have a system of three pairs of glands, in which by following the Townley et al. (1993) model, each silk gland will function at a different time during the molting cycle. In general, major ampullate silk glands have three distinct parts: a secretory tail (Fig. 1A, star), an ampule-shaped storage sac, and an elongated spinning duct (Fig. 1A, arrow) (Vollrath & Knight 1999; Chaw & Hayashi 2018). In *D. spinosa*, all major ampullate silk glands have an ampule-shaped sac and an elongated spinning duct, with the three longest glands (Fig. 1A, left group) also having a long tail similar to other species (Vollrath & Knight 1999; Clarke et al. 2017; Chaw & Hayashi 2018). The other seven major ampullate silk glands have shorter tails, tend to be clustered together, and are smaller, approximately one-fourth the size of the longest glands (Fig. 1A, right group). We also found differences in the size of the ampullate spigots on the ALS (Fig. 2A) associated with these glands: there are three larger spigots on the inner side closer to the piriform spigots and a cluster of seven smaller major ampullate spigots on the outer side (Fig. 2B). Given the presence of additional glands in juvenile spiders (data not shown), the current understanding of molt-related changes in silk glands (Townley et al. 1993), and our observation that all major ampullate glands are filled with silk dope, we posit that all ten major ampullate silk glands found in mature *D. spinosa* are fully functional. Whether the morphological differentiation in *D. spinosa* major ampullate silk glands translates into usage or functional diversification of major ampullate fibers is unclear.

Historically, minor ampullate silk glands were named due to their morphological similarity to major ampullate silk glands, albeit smaller in size. In *D. spinosa*, the minor ampullate silk glands were not found to be ampule-shaped, as in other orb-weaving spiders, but instead were identified based on their location, by following their ducts to the minor ampullate spigots on the anterior median edge of each posterior median spinneret (PMS; Fig. 2A, D). We observed only one minor ampullate spigot on each PMS. Moreover, we found no evidence of a second pair of minor ampullate spigots. By contrast, Peters (1992) found two ampullate spigots in *Asianopis subrufus* (L Koch, 1878) (recently transferred from *Deinopis subrufa*): a large one corresponding to minor ampullate and a smaller one that was difficult to observe. Following Peters (1992), Griswold et al. (2005) reported *Deinopis* as having two minor ampullate spigots, although only one minor ampullate spigot was visible in their micrographs of *Deinopis*. It may be that *D. spinosa* and *A. subrufa* differ in the number of minor ampullate silk glands. In *D. spinosa*, minor ampullate silk glands are bifurcated in the sac (or secretory section), have a very long secretory duct, and a short tail compared to major ampullate silk glands (Fig. 1B). Bifurcation of minor ampullate silk glands has also been observed exclusively in other cribellate spiders (Řezáč et al. 2017), suggesting that bifurcation could be functionally paired with the production and/or function of the undulating fibers in cribellate silk.

The other silk glands not used in cribellate capture threads are the aciniform, piriform, and tubuliform (also known as cylindrical) silk glands. We found the aciniform and piriform silk glands to be among the smallest gland types (Fig. 1C, D). The aciniform silk glands lack morphologically distinct tails and were tightly packed in a grape cluster-shaped formation (Fig. 1C). There were two pairs of clusters (one on each side): a cluster of ~47 aciniform glands attached to the posterior lateral spinneret (PLS) and a cluster of ~96 aciniform silk glands attached the PMS (Fig. 1C). Moreover, morphological differences in aciniform silk glands and spigots in *D. spinosa* were not observed as with other spiders (Peters & Kovoor 1980; Kovoor & Peters 1988).

Piriform silk glands were also tightly clustered, with ~100 glands arranged in a "baseball-glove" shape (Fig. 1D). It was not possible to separate individual aciniform or piriform silk glands without destroying them. Both aciniform and piriform silk glands were found in the hundreds and have very small and thin ducts. Tubuliform silk glands were long, with a yellow-orange color, and take on the previously described noodle-shape of tubuliform glands from other Entelegynae species (Fig. 1E). We found the tubuliform silk glands to be extraordinarily numerous, ~90 pairs, compared to araneoid spiders, which possess only three pairs of tubuliform glands (Coddington 1989; Griswold et al. 1998, 2005; Murphy & Roberts 2015). Deinopids construct very densely woven and hard egg cases (Barrantes et al. 2014), and these properties likely result from their large number of tubuliform glands.

Prey capture silk in deinopids is a composite of multiple components, including the ultrafine cribellar fibrils, the extensible core pseudoflagelliform silk fibers and connective paracribellate fibers (Peters 1984, 1992). A fourth component of deinopid cribellate silk is minor ampullate silk which is used in the undulating fibers as mentioned above (Řezáč et al. 2017). The morphologies of the glands associated with pseudoflagelliform and paracribellate silks are largely unknown for any species. We identified a single noodle-shaped silk gland attached to a single large spigot present on the lateral edge of the PLS (Fig. 2A,C). This spigot has been considered to be homologous to the flagelliform silk spigots of ecribellate orb-web weavers because of similarity in position and shape and was thus called the pseudoflagelliform spigot (Coddington 1989; Peters 1992; Alfaro et al. 2018). It follows that the silk gland attached to this spigot is the pseudoflagelliform silk gland (Fig. 1F). Like the flagelliform silk gland, the pseudoflagelliform silk gland is elongated ampule-shaped, with a distinct tail and a small kink towards the duct (Fig. 1F). As for the second component of cribellate silk, the paracribellate silk glands were identified by tracing the glands to the corresponding spigots on the PMS (Fig. 2D). The paracribellate silk glands are ampule-shaped with thin, elongated ducts and short tails. Paracribellate glands are numerous, at ~120 (~60 silk glands on each side), tightly arranged in clusters (Fig. 1G), and separating them was challenging (but see Fig. 1H). The final silk type, cribellar silk glands, are attached to a special plate-like organ called the cribellum (Fig. 1I, brown line), which is an identifying feature of cribellate spiders. The cribellar silk glands were so snugly clustered together, it was impossible to isolate a single gland (Fig. 1I). The number of glands was also so vast that quantification was not possible with the standard light microscopy techniques used here. However, a study of *A.subrufa* estimated that there were ~25,000 spigots on the cribellum, suggesting ~25,000 cribellar silk glands (Peters 1992). Histological studies show that cribellar glands are arranged as a compact mass widespread above and around the PLS and PMS (Kovoor & Peters 1988), which is consistent with the morphology and the number described in this work. When the cluster of *D. spinosa* cribellar glands was torn apart, thin ducts were observed and the glands appeared to be spherical, with no visible tails, an observation consistent with early illustrations by Bertkau (1882). The glands were arrayed in tightly packed rows that folded back on themselves, giving the appearance of a box pleated skirt.

We show for the first time the morphology of the complete complement of silk glands from a cribellate spider. Moreover, we describe the previously unknown morphology of the paracribellate, pseudoflagelliform, and cribellar silk glands. Confirmation of the presence of a pseudoflagelliform gland in a deinopid, exhibiting morphological and positional similarity to araneoid flagelliform glands has special significance for our understanding of spider silk evolution and the origin of the iconic orb-web. Early debates centered on whether the orb-web evolved convergently in different spider lineages or if it had a single origin (Shear 1986). Coddington (1989) proposed homology of the pseudoflagelliform and flagelliform silk spigots, implying that cribellate spiders spinning orb-webs and orb-web derivatives (Uloboridae and Deinopidae) were the sister group of Araneoidea (ecribellate orb-weavers), and that the orb-web traced to a single origin in their most recent common ancestor. However, recent phylogenomic reconstructions of the spider tree of life are contentious: several have repositioned deinopids, uloborids, and araneoids in different ways; and they disagree as to whether the orb-web had a single, but more ancient origin (e.g., Garrison et al. 2016; Coddington et al. 2019), versus multiple, convergent origins (e.g., Fernández et al. 2018; Kallal et al. 2020). Adding to this, (Eberhard 2022) recently argued that a suite of several web-building behaviors shared across cribellate and ecribellate orb-weavers are homologous, and strongly favor a single origin of this web architecture. Like these behavioral characters, the positional and morphological similarity of the pseudoflagelliform and flagelliform silks glands in deinopids and araneoids, reinforces that they are homologous (Alfaro et al. 2018; Correa-Garhwal et al. 2022). Given that silk extruded from these glands is predominantly used in the orb-web capture spiral (for both cribellate and ecribellate orb-weavers) or in deinopid capture silk supporting fibers, resolution of the phylogenetic positions of these spider lineages will not only inform the number of times the orb-web evolved, but also the number of times the pseudoflagelliform/flagelliform glands have been lost, as well as how silk fibers extruded from their spigots have functionally transformed over evolutionary time. Beyond the importance of the pseudoflagelliform gland, future studies are needed to understand the function and evolution of the entire suite of silk glands that allow deinopids to capture prey with their remarkable, hyper-extensible capture nets.

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