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Phil. Trans. R. Soc. Lond. B 1993 **340**, 25-38
doi: 10.1098/rstb.1993.0046

Moult-related changes in ampullate silk gland morphology and usage in the araneid spider *Araneus cavaticus*

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SUMMARY

Major ampullate (MaA) and minor ampullate (MiA) silk glands of juvenile *Araneus cavaticus* (third to penultimate instars) were examined by dissection at various times relative to ecdysis. Several days before ecdysis the larger pairs of MaA and MiA glands become non-functional and remain so until ecdysis. Nevertheless, proecdysial spiders are able to draw ampullate fibres due to the presence of smaller pairs of MaA and MiA glands which are functional at this time. Indeed, it appears that these smaller ampullate glands are intended for use only during proecdysis. Thus, larger MaA and MiA glands and smaller MaA and MiA glands are typically not used concurrently (a brief transitional period is an exception). The smaller ampullate glands functioning in one juvenile stadium regress in the following stadium and become (what have previously been referred to as) accessory MaA and MiA glands. These non-functional accessory ampullate glands do not re-develop into functional smaller ampullate glands until the following stadium. Thus, a given pair of smaller MaA or MiA glands is only functional in every other juvenile stadium. However, because there are two sets of smaller/accessory MaA and MiA glands which function alternately, the spider is able to produce ampullate fibres during the proecdysial portion of each stadium. A new terminology for the larger, smaller and accessory ampullate glands is proposed which emphasizes the kinship between the two sets of smaller/accessory ampullate glands.

1. INTRODUCTION

As first described by Sekiguchi (1955*b*), juveniles of species within the araneid and tetragnathid spider families typically, if not always, have two, clearly identifiable pairs of major ampullate (MaA) silk glands and spigots and two pairs of minor ampullate (MiA) silk glands and spigots, with one pair of each gland type being larger than the other. Following the last moult the smaller pair of each type is lost, as are the spigots serving these glands. These statements apply to both sexes. Based on observations made on *Araneus cavaticus* (Araneidae), we reported that the ampullate glands that are lost are not totally resorbed but atrophy until only small, often yellowish, structures remain (Townley *et al.* 1991). Such structures were first observed by Johansson (1914) and termed accessory (ampullate) glands. In addition to the two pairs of accessory ampullate glands which result from the degeneration of ampullate glands functional in the penultimate stadium, there are two pairs of accessory ampullate glands (one MaA pair and one MiA pair) present in juveniles, starting at least with third instars (Townley *et al.* 1991). (Although first and second instars have not been examined internally, if the conclusions drawn in this paper are correct, then the two pairs of juvenile accessory ampullate glands should be present beginning at least with first instars which are approaching the end of the interecdysial period.) These juvenile accessory ampullate glands are also retained in adults, giving them a total of four pairs of accessory ampullate glands.

In our earlier report (Townley *et al.* 1991), we could not explain the presence of the juvenile accessory ampullate glands. However, we did propose that the smaller pairs of MaA and MiA glands are present in juveniles, but not in adults, because they function during the period preceding each ecdysis when the larger MaA and MiA glands are temporarily non-functional, assuming the duties of the larger glands and allowing the spider to produce ampullate fibres throughout the moulting process. Once adulthood is reached no further moults occur, making the smaller MaA and MiA glands unnecessary. In the present communication we present additional evidence which corroborates this interpretation. We also propose that the accessory ampullate glands in juveniles are vital components of this system for ensuring continuity of ampullate silk production. Specifically, we propose that the accessory MaA and MiA glands present at one juvenile stadium become the smaller MaA and MiA glands of the following juvenile stadium and vice

Phil. Trans. R. Soc. Lond. B (1993) **340,** 25–38
Printed in Great Britain

versa. This implies that the smaller MaA and MiA glands and their accessory gland counterparts are essentially identical entities which are simply at different points in a shared cycle of development and regression.

2. MATERIALS AND METHODS

First to ninth instar (juvenile) *Araneus cavaticus* (Keyserling) of both sexes were collected in southeastern New Hampshire between April and September. The same parameters (carapace dimensions, the number of aciniform fusules on each posterior median spinneret (PMS), the position of the MiA nubbin on each PMS) that were previously used to assign juvenile spiders to specific stadia (Townley *et al.* 1991) were again used in the present inquiry. Although our efforts have been focused on *A. cavaticus*, for one specific purpose it proved valuable to examine two other araneid species, *Argiope aurantia* Lucas and *Argiope trifasciata* (Forskål) (see § 4*c*). Late juvenile females and adult males and females of these species were also collected locally, from July to August (*A. aurantia*) or August to September (*A. trifasciata*).

Ampullate glands and the spinnerets on which their ducts empty were dissected from opisthosoma and examined with an Olympus model X-Tr stereo dissecting microscope and a Nikon Fluophot microscope. For examinations on the latter microscope, non-permanent whole mounts of spinnerets and portions of ampullate glands were prepared and viewed with regular brightfield illumination. Spinnerets (from both freshly killed spiders and exuvia) and ampullate glands were also examined by scanning electron microscopy (SEM) using the same methods described previously (Townley *et al.* 1991). Internal components of the spinning apparatus were not examined in first or second instars. A Ringer's solution consisting of 160 mM NaCl, 7.5 mM KCl, 4 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $NaHCO_3$ and 20 mM glucose, pH 7.4 (Groome *et al.* 1991), was used during dissections.

To determine which ampullate glands were being used by a particular spider at a known time after ecdysis, the paraffin technique described by Peters (1982) was used. In this procedure the application of hot, liquid paraffin to the spinnerets allows emerging fibres to remain intact while the spinnerets are being processed for SEM. However, rather than adding paraffin to the spinnerets of living spiders, as in the original procedure, in this study paraffin was applied only after spiders had been anesthetized and severed at the pedicel and the spinnerets had been excised and mounted on modified pin-type SEM stubs (Townley *et al.* 1991). Of course, although care was taken to neither initiate new fibres after death nor sever fibres which were present before the spiders were anesthetized, the original procedure is preferable. The modification was made nevertheless because it was desirable to determine both the ampullate glands that were in use and the condition of the ampullate glands in the same individual. Thus, the spinnerets had to be excised while leaving the ampullate glands as intact as possible. This was more readily accomplished by applying paraffin to the spinnerets after they had been removed from the rest of the opisthosoma.

The spiders used to make these determinations were collected in the field and reared in rectangular cages in the laboratory (Tillinghast & Kavanagh 1977) until they ecdysed. They were killed at various times after ecdysis ranging from immediately after the removal of the legs from the exuvium to 32.6 days. A strict feeding regimen was not imposed on the spiders between ecdysis and death, but they were given one fly (primarily *Phaenicia sericata* (Meigen), *Phormia regina* (Meigen) and *Musca domestica* Linnaeus) on each day that they had an orb web which was at least two-thirds intact. All spiders were given water daily.

3. TERMINOLOGY

There is presently no agreement on the terminology and definitions used to describe the embryonic and postembryonic stages of spiders, and we are in no position to resolve the matter. For the purposes of this paper it is sufficient that we be consistent. Thus, as in our earlier report (Townley *et al.* 1991), a starting point was established by designating the earliest instar of *A. cavaticus* collected in the field the first instar. This instar has two aciniform fusules on each PMS and, more importantly, no MiA nubbins and is almost certainly the instar which emerges from the eggcase. Successive instars were then numbered accordingly from this starting point.

Previously, we distinguished those ampullate glands that are functional in both juveniles and adults from those that are functional only in juveniles with the adjectives 'larger' and 'smaller', respectively (Townley *et al.* 1991); a reference to the difference in size between these glands. Thus, we recognized larger and smaller MaA glands and larger and smaller MiA glands. The small, vestigial-looking ampullate glands in juveniles and adults were, by precedent (Johansson 1914), termed 'accessory' MaA and MiA glands. Part of our aim in writing this paper, however, is to present evidence which indicates that 'smaller' and 'accessory' ampullate glands exchange identities after each ecdysis (except the last) and should, therefore, receive a common nomenclature to reflect their common function and nature. Accordingly, throughout the remainder of this paper we will distinguish those ampullate glands that function (i.e. give rise to ampullate fibres) in both juveniles and adults from those that function only in juveniles by use of the terms 'primary' (1°) and 'secondary' (2°), respectively. 'Secondary' in the new terminology encompasses both 'smaller' and 'accessory' in the old terminology. Although it is important to stress the commonalities between 2° ampullate glands, it is still useful to be able to distinguish those 2° ampullate glands which will be or are functioning in a given stadium from those which cannot function in that stadium. The morphological feature which distinguishes these two categories of 2° ampullate glands throughout an interecdysial period is the presence or absence of an outlet for a gland's duct to the outside (i.e. a spigot which opens to the outside environment). When referring to a 2°

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ampullate gland that has such an outlet in a given stadium we will use the term 'open'. Conversely, 2° ampullate glands lacking an outlet are 'blocked'. It is important to keep in mind that the term 'ampullate glands' includes both MaA and MiA glands (e.g. open 2° ampullate glands includes open 2° MaA glands and open 2° MiA glands).

4. RESULTS

In the following description of moult-related changes in the ampullate glands of *Araneus cavaticus*, there is, of necessity, some overlap with observations presented by us previously (Townley *et al.* 1991). In addition to examining the figures in the present paper, the reader may find it useful to refer to figures in the earlier paper while reading the following. Note that while the overall course of events described below has been observed in juveniles starting with third instars, the approximate times given are taken from observations made on seventh to ninth juvenile instars.

(*a*) *Overt changes in ampullate glands associated with the moult cycle*

In juvenile *A. cavaticus* which are about midway between two consecutive ecdyses, the ampullae and tails of the 1° MaA and MiA glands are translucent and contain relatively large quantities of luminal material, resulting in glands with a turgid aspect. Their ducts have a sharply delineated, crystalline appearance which is, presumably, a consequence of a highly ordered cuticular intima (Bell & Peakall 1969; Kovoor & Zylberberg 1972; Moon *et al.* 1988; Moon & Kim 1989). The open 2° MaA and MiA glands are considerably smaller than the 1° ampullate glands and have much shorter tails, but otherwise they are similar in their gross morphology. However, at this time their ampullae are typically not as distended with luminal contents as they will become later in the interecdysial period. The blocked 2° MaA and MiA glands are small and very thin at this time with no discernible luminal contents. Their ducts are 'non-crystalline' and vestigial and clearly would not be functional even if they were not 'blocked'.

As juvenile *A. cavaticus* approach ecdysis (approximately 3.5–6 days before ecdysis) the 1° MaA and MiA glands enter a white opaque phase in which the translucent epithelium of the ampulla/tail gradually becomes more of an opaque white and has a more granular mien. The amount of transparent, gel-like luminal material is concurrently reduced until, at the height of this phase, it is no longer possible to observe such luminal contents by dissection and the ampullae are flaccid. Likewise, the functional, crystalline appearance of the ducts is gradually lost and replaced by a similar fuzzy, white opacity. The point at which the 1° ampullate glands become non-functional is seemingly determined by the changes occurring in the ducts as transparent luminal contents remain in the ampullae/tails beyond the time at which the ducts become (to the eye) non-functional. No such changes occur in the open 2° MaA or MiA glands at this time. Quite the contrary, these glands reach their peak at about this time, in terms of overall size and the amount of aqueous polypeptide solution accumulated in the ampulla's lumen (see figures 2–4 in Townley *et al.* (1991)). When the spider is within about 1–1.5 days of ecdysing, the 1° ampullate glands show signs that they are returning to their former condition. Typically, their ducts first begin re-forming and then, shortly after, the familiar transparent luminal contents start to re-accumulate. As the ducts re-form, 'crystallinity' gradually returns while opacity is reduced. Approximately concurrent with the re-formation of the 1° ampullate gland ducts is the similar re-formation (or formation, in first instars, presumably) of the ducts of the blocked 2° ampullate glands.

Solely on the basis of external appearance, it would seem that the 1° ampullate gland ducts are almost fully re-formed when ecdysis occurs. Only that portion bordering on the ampulla is not, and it is not until about 2–3 days after ecdysis that the sharply demarcated, distinctive appearance of this part of the duct is fully restored. The epithelium of the ampulla/tail is still somewhat opaque at the time ecdysis occurs but a return to a more translucent condition is clearly in progress. Also at this time, at least a moderate amount of luminal material is present and this also increases the translucence of the 1° ampullate glands. Immediately preceding ecdysis the ampullae and tails of the blocked 2° ampullate glands are not much wider than they were midway in the interecdysial period, but a lumen containing a very small amount of fibroin is usually discernible. More significant is the observation that normal, ampullate-type ducts have, to a large extent, formed by this time. However, their crystalline aspect, so striking in the ducts of the open 2° ampullate glands at this time, is only partially developed. The well developed and fully functional appearance of the open 2° MaA and MiA glands is maintained throughout proecdysis.

Following ecdysis, the new open 2° ampullate glands (i.e. the blocked 2° ampullate glands of the preceding stadium) increase in size as luminal contents are amassed, while the new blocked 2° ampullate glands regress. Because both of these processes take place gradually, the blocked 2° ampullate glands are clearly more prominent than the open 2° ampullate glands in the first hours after ecdysis. It is for this reason that the terms 'smaller ampullate glands' and 'accessory ampullate glands', in our old terminology, are usually, but not always, synonymous with 'open 2° ampullate glands' and 'blocked 2° ampullate glands', respectively, in our current terminology. Immediately after ecdysis, those glands which we would have identified previously as smaller ampullate glands, we now know to be 'blocked', and those which we would have identified as accessory ampullate glands, we now know to be 'open' (see figure 5*c*, *f*). By about 1.5–2.5 days after ecdysis, sufficient regression and development have typically occurred in the blocked and open 2° ampullate glands, respectively, that their ampullae have similar diameters. However, the appearances of these glands are considerably different at this time; the blocked 2° ampullate glands having fuzzy, white

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ampullae and tails with little or no luminal contents and thin, delicate, 'non-crystalline' ducts, while the open 2° ampullate glands have translucent ampullae and tails containing still fairly small quantities of luminal material and seemingly functional, 'crystalline' ducts. Within about 3–3.5 days after ecdysis, the blocked 2° ampullate glands have regressed to the point where they possess those characteristics which were formerly used to identify accessory ampullate glands; very thin ampullae and tails, no transparent, gel-like luminal contents discernible by dissection, and vestigial ducts. These glands do not begin their return to a functional condition until shortly before the next ecdysis, as described above. Because 2° ampullate glands are not needed after the final moult, the development which takes place in the blocked 2° ampullate glands of prepenultimate instars shortly before ecdysis (in preparation for their use in the following stadium) does not occur in penultimates.

As indicated above, the open 2° ampullate glands of a given stadium largely retain their proecdysial appearance for about 6–12 hours into the following stadium (in which they are 'blocked'). However, immediately after ecdysis, the ducts of these new blocked 2° ampullate glands, which appear crystalline and functional over most of their length, are usually found to end abruptly about one-half to three-quarters of the way into the spinnerets.

When functional, the ducts of all six pairs of ampullate glands follow a zig-zagging course, as is typical of ampullate gland ducts (see Meckel 1846; Atanasiu–Dumitresco 1941; Sekiguchi 1952; Mullen 1969; Kovoor 1977, 1979). Starting at the ampulla, the duct proceeds posteriorly, but after traversing much of the distance to the spinnerets, it makes a hairpin turn and travels anteriorly, running more or less parallel to the first segment. It then makes another hairpin turn and travels to the spinnerets, again running parallel to the first two segments. In the case of the MiA and 2° MaA glands, the duct makes the second hairpin turn before reaching the ampulla–duct junction (exceptions have been seen in 1° MiA glands where the second turn can occur at or slightly beyond the ampulla–duct junction). In the 1° MaA glands, the duct passes the ampulla–duct junction before making the second turn and that portion of the duct which is anterior to the ampulla–duct junction forms a loop which lies appressed to the side of the ampulla (see plate V, figure 1 in Atanasiu-Dumitresco (1941); figure 1 in Kovoor & Zylberberg (1972); figure 7 in Townley *et al.* (1991)). When 1° ampullate glands are entering the white opaque phase or when blocked 2° ampullate glands are regressing shortly after an ecdysis, their ducts take on a fuzzy, whitish or yellowish appearance. At the height of regression the ducts' serpentine course is no longer discernible and, thus, fully regressed ducts bear little resemblance to functional ducts.

(*b*) *Connections between ampullate gland ducts and spigots in juveniles*

During most of an interecdysial period (from immediately after ecdysis to shortly after apolysis) it can be observed that the ducts serving the 1° MaA glands exit on the more anterior of the two pairs of MaA spigots, whereas the open 2° MaA gland ducts exit on the posterior MaA spigots. Likewise, the 1° and open 2° MiA gland ducts exit on the anterior and posterior MiA spigots, respectively. The blocked 2° ampullate gland ducts are atrophied during most of this interval and do not connect to any spigots.

In the interval which begins with the emergence from the white opaque phase (i.e. when 1° and blocked 2° ampullate gland ducts are re-forming) and ends with ecdysis, the 1° ampullate gland ducts again lead to the more anteriorly-placed ampullate spigots, but on the newly developing cuticle. There is no connection made to the overlying old cuticle (figure 1). (A segment of duct can sometimes be seen attached to the 1° ampullate spigots on the old cuticle, but it travels back from the spigot only a short distance before ending.) The ducts of the well developed, open 2° ampullate glands, in contrast, pass through the new cuticle and maintain their connections to the 2° ampullate spigots on the old cuticle (figures 1, 2). (Here and subsequently it should be borne in mind that it is actually the new cuticle which must form around the open 2° ampullate gland ducts, as the latter are in place long before new cuticle is laid down.) Connected to the 2° ampullate spigots on the new cuticle are the ducts of the blocked 2° ampullate glands (figure 1).

Penetration of the new cuticle by the ducts of the open 2° ampullate glands occurs at specific sites. In the case of the open 2° MiA glands, their ducts pass through the new cuticle at one of two sites; these being between the two MiA spigots on a given PMS (figure 3*a*) and posteromedial to the 2° MiA spigot (figure 3*b*, also figure 1). In any given stadium, the site of penetration is the same on both of the PMS. Alternation between these two sites occurs from one stadium to the next. The 2° MiA spigots likewise occupy one of two sites in a given stadium and it is clear that they are coincident with the two potential sites of 2° MiA duct penetration. Thus, 2° MiA spigots occupy the site which is not occupied by penetrating open 2° MiA ducts during the cuticle's formation. The ducts of the open 2° MaA glands, on the other hand, pass through the new cuticle at the same site in all stadia; this being lateral to both MaA spigots on an anterior lateral spinneret (ALS) and, typically, closer to the 2° MaA spigot (figure 3*c*, also figure 2*b*).

Once ecdysis occurs, the outlets (i.e. spigots) for the open 2° ampullate glands of the preceding stadium are, of course, removed and these glands now enter a regressive period as blocked 2° ampullate glands. An observation made on exuvia indicates that the segment of open 2° ampullate gland duct positioned between the old and new cuticles before ecdysis is lost with the old cuticle at ecdysis. When exuvia shed by first through penultimate instars are examined, silk fibres are often found passing through the 2° ampullate spigots (but never through the 1° ampullate spigots) (figure 4). That portion of a fibre on the 'internal' side of the exuvium can usually be seen to

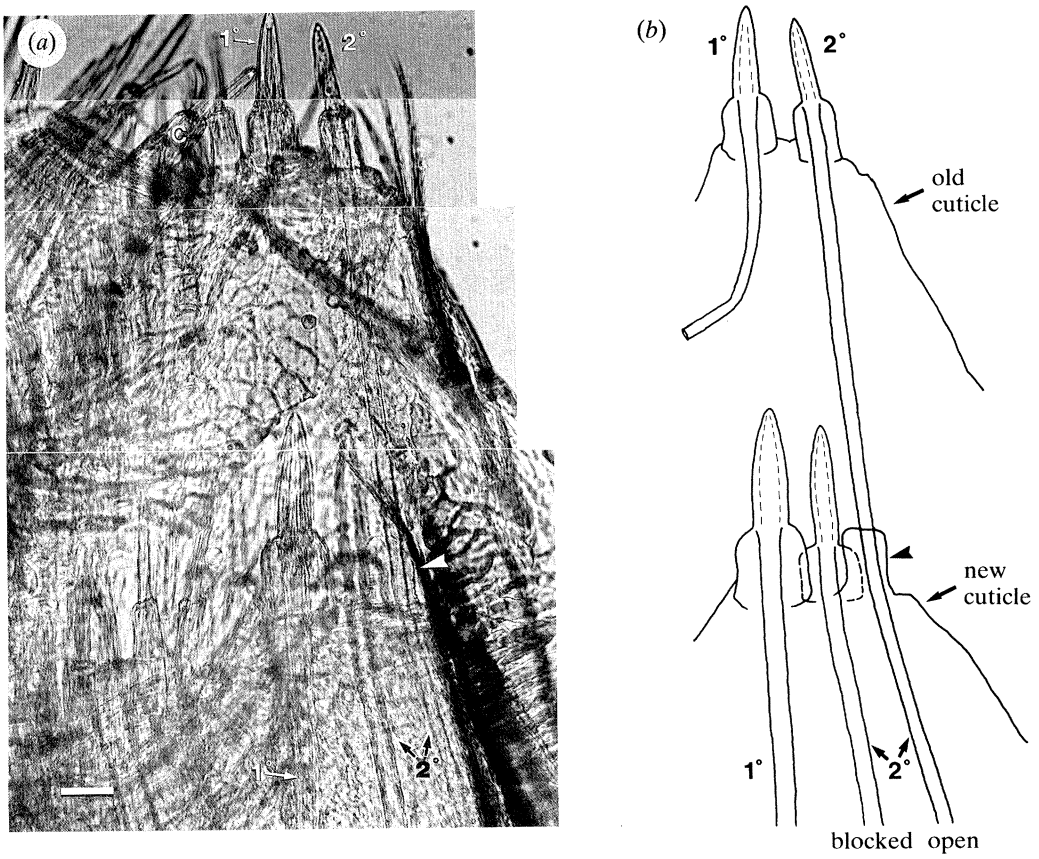

Figure 1. (*a*) A portion of the left PMS from a seventh instar female *A. cavaticus*, close to ecdysing, showing the new cuticle beneath the old cuticle. The diagram in (*b*) outlines those structures in the photomicrograph relevant to the topic at hand. Emergence from the white opaque phase was well under way by this time, so that the largely re-formed duct of a 1° MiA gland is clearly visible connecting to the more anterior MiA spigot on the new cuticle. Only once the old cuticle was removed at ecdysis would this gland have been able to resume functioning. A portion of duct is faintly visible connecting to the 1° MiA spigot on the old cuticle; a remnant, presumably, of the duct which was functioning prior to the white opaque phase. The partially re-formed duct of a blocked 2° MiA gland can be seen attached to the more posterior MiA spigot on the new cuticle. This gland, very thin at this time,would not have been used until late in the following stadium. Connected to the 2° MiA spigot on the old cuticle and passing through the new cuticle is the duct of a well-developed, open 2° MiA gland. This gland would have remained functional until ecdysis. Note that the duct passes through a protuberance (nubbin progenitor) in the new cuticle (arrowheads in (*a*) and (*b*)). The unidentified spinning structures to the left of the MiA spigots in (*a*) are aciniform fusules. C, cylindrical gland spigot. Bar = 25 μm.

have a sheath over its more proximal end (relative to the exuvium). We presume this sheath is the dried remains of a segment of duct (at least its cuticular intima). The length of this sheath leads one to consider the possibility that not only the segment between the two cuticles is lost at ecdysis, but that a length of duct internal to the new cuticle is also withdrawn at ecdysis. The observation that blocked 2° ampullate gland ducts end part way into the spinnerets in freshly ecdysed spiders is consistent with this notion.

As mentioned above, the newly re-formed ducts of blocked 2° ampullate glands are connected to the 2° ampullate spigots on the new cuticle prior to ecdysis. With the removal of the old cuticle at ecdysis these spigots are opened to the environment and, thus, the glands connected to them are now, by our definition, open 2° ampullate glands. Removal of the old cuticle also exposes the spigots to which the 1° ampullate glands are connected.

The mechanism by which ampullate fibres are drawn during each proecdysial period is summarized in figure 5.

(*c*) *Nubbin placement*

Evidence of the passage of 2° ampullate gland ducts through a developing cuticle remains in the cuticle after ecdysis in the form of protuberances, termed nubbins in Coddington (1989) and Yu & Coddington (1990). In juvenile *Araneus cavaticus*, MiA nubbins are relatively easy to locate, on both exuvia and the spiders themselves. In an examination of 25 spiders (15 male, 10 female) and one or more of the most recently shed exuvia from these individuals (a total of 56 exuvia were examined, shed by first to ninth

instars), MiA nubbins and 2° MiA spigots were seen to switch positions with each other from one stadium to the next, in both sexes and without exception. In even-numbered juvenile instars the MiA nubbins are placed posteromedial to the 2° MiA spigots whereas in odd-numbered juvenile instars the MiA nubbins are located anterolateral to the 2° MiA spigots (except in first instars, which have no MiA nubbins (Yu & Coddington 1990; Townley *et al.* 1991)) (see figures 25–32 in Townley *et al.* (1991)).

To date, we have not been able to convincingly observe MaA nubbins in juvenile *A. cavaticus* (although in a few specimens, portions of structures very suggestive of MaA nubbins could be observed). This is, perhaps, not surprising when one considers the location at which open 2° MaA gland ducts pass through the developing cuticle (shortly before ecdysis) and the proximity of the pyriform spinning field to the lateral sides of the MaA spigots. That is, the MaA spigots on an ALS reside on a depressed ledge which is bordered anteriorly, laterally and posteriorly by the pyriform spinning field. We believe we have been unable to clearly view MaA nubbins in juveniles because they are obscured by a portion of the pyriform spinning field which extends over or compresses the area where MaA nubbins would be expected. On the chance that the precise morphology of this area might not be the same in all araneids, two *Argiope* species (*A. aurantia* and *A. trifasciata*) were also examined for MaA nubbins. In both species, a greater separation between the edge of the pyriform spinning field and the lateral sides of the MaA spigots is indeed present, and the boundary between MaA ledge and pyriform spinning field more reinforced, allowing one to observe, in the position expected, a single MaA nubbin on each ALS in juveniles (figure 6*a*). (By using proecdysial, penultimate female *A. aurantia* and *A. trifasciata*, it has been directly observed that the site of open 2° MaA gland duct penetration through the developing cuticle is essentially the same in these two species as in *A. cavaticus*.)

Because there is no need for the blocked 2° ampullate glands of penultimates to re-develop in adults, there is also no need for 2° ampullate spigots to form in the adult cuticle. Only nubbins occur at the sites where 2° ampullate spigots would have been present, if the animal was not an adult. These nubbins differ from those described above in juveniles in that they do not result from the passage of 2° ampullate gland ducts through the cuticle during its formation. Nor, apparently, do they arise from a template (either 2° MaA spigot or MiA nubbin) in the overlying, penultimate cuticle, as the exuvial and developing cuticles are not in direct contact, but are separated by an

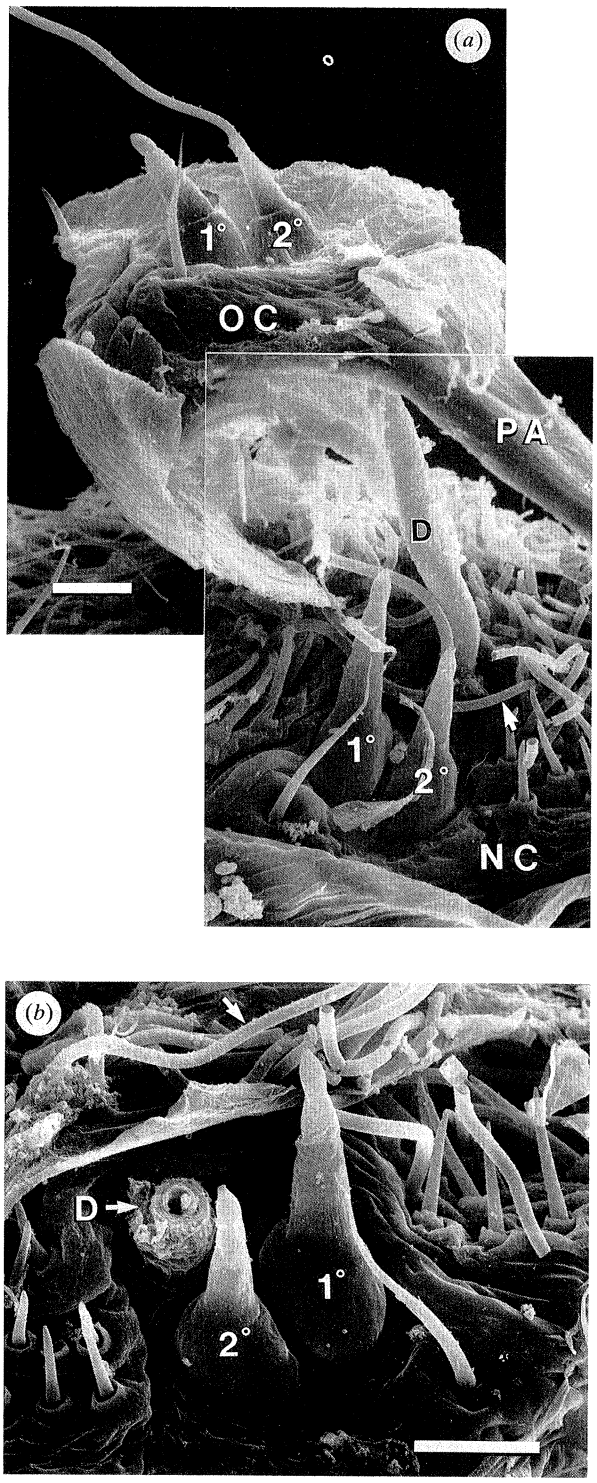

Figure 2. Seventh instar male *A. cavaticus* in proecdysis: (*a*) Part of the left ALS showing the 1° and 2° MaA spigots on both the old cuticle (OC) and the underlying, new cuticle (NC). The duct (D) of an open 2° MaA gland can be seen passing through the new cuticle to maintain its attachment to the 2° MaA spigot on the old cuticle, thus allowing the spider to continue drawing MaA fibres during proecdysis. (*b*) Part of the right ALS from the same individual, showing, more precisely, the site at which open 2° MaA gland ducts penetrate the new cuticle in proecdysial spiders. Only the new cuticle is shown. The duct (D) of the open 2° MaA gland has been broken off just distal to the new cuticle. In (*a*) and (*b*) tubules can be seen scattered on the surface of the new cuticle (unlabeled arrows point to examples). These tubules are portions of pyriform gland ducts which, like the open 2° MaA gland ducts, were passing through the new cuticle to connect to fusules on the old cuticle (and were severed during dissection). PA, principal apodeme (see Wilson 1969). Bars = 25 μm.

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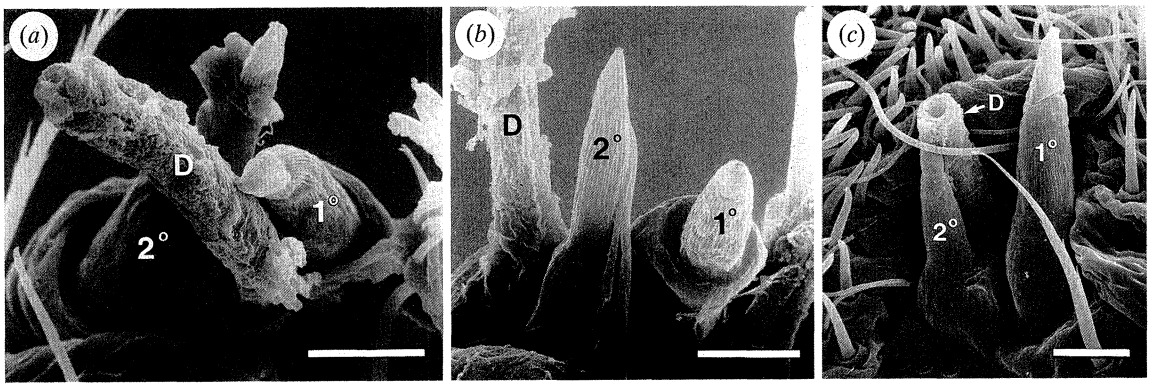

Figure 3. 1° and 2° MiA (*a*,*b*) and MaA (*c*) spigots on the new (developing) cuticle of proecdysial *A. cavaticus*. (*a*) Left PMS from an eighth instar male, (*b*) left PMS from a seventh instar male, (*c*) right ALS from a sixth instar female. The overlying, old cuticle has been removed in these preparations (and the ducts of the open 2° ampullate glands severed from the old cuticle). In even-numbered instars (*a*) the ducts of the open 2° MiA glands pass through the new cuticle anterolateral to the 2° MiA spigots whereas in odd-numbered instars (*b*) the ducts pass posteromedial to the 2° MiA spigots. Open 2° MaA ducts penetrate the new cuticle lateral to the 2° MaA spigots in both even-numbered (*c*) and odd-numbered instars (see figure 2*b*). D, duct of open 2° ampullate gland. Bars = 25 μm.

exuvial space. Rather, they seem to be vestigial spigots, aborted early in their formation. Adults also have ampullate nubbins of the type found in juveniles, although, again, the MaA nubbins of this type have been clearly observed only in the two *Argiope* species (figure 6*b*), not in *A. cavaticus*.

(*d*) *Ampullate gland usage*

Using the paraffin technique of Peters (1982), we determined which ampullate glands were being used by juveniles at various times after ecdysis. The results for MaA glands are shown in figure 7. The mean feeding rates for individual spiders ranged from 0.32–0.89 flies per day ($\bar{x} = 0.55$ flies per day, SEM = 0.023 flies per day, $n = 35$) (excluding those spiders killed within 2 days after ecdysis). In all cases, the same MaA glands were in use on both the right and left sides of the opisthosoma. Two of the 41 spiders examined showed fibres exiting simultaneously on the 1° and 2° MaA spigots. In the remaining 39 spiders, fibres were seen emerging from either the 1° MaA spigots exclusively or the 2° MaA spigots exclusively. During the first (and largest) portion of an interecdysial period (beginning immediately after ecdysis, while the spider is still hanging by its molting threads) 1° MaA fibres are drawn, while in the latter portion 2° MaA fibres are drawn.

More complicated results were obtained for the MiA glands. In 14 of the 41 spiders, the emergence of fibres from the MiA spigots on both PMS was in complete agreement with the MaA situation. Fifteen of the 41, on the other hand, had no MiA fibres present at all. The members of these two groups were not distributed in any evident pattern. Three individuals which were using their 1° MaA glands showed no MiA fibres on one PMS, but had a fibre emerging from the 1° MiA spigot on the second PMS. Another spider with 1° MaA glands in use had no MiA fibres on one PMS, but had MiA fibres exiting on both the 1° and 2° MiA spigots on the other PMS. Still another spider which was using its 1° MaA glands had a 1° MiA fibre on one PMS and 1° and 2° MiA fibres on the second PMS. Further, while preparing the spinnerets of one spider, the apex of one PMS was inadvertently touched with a fine insect pin, initiating a MiA fibre. Upon inspection by SEM, 1° MaA fibres were observed, as was a single 2° MiA fibre, drawn posthumously. Of the two spiders that had fibres emerging from both the 1° and 2° MaA spigots, one fell into the aforementioned category of spiders in which the MaA and MiA situations were in complete agreement, while the second spider had 1° and 2° MiA fibres emerging from one PMS, but only a 1° MiA fibre on the other PMS. Of particular interest was one spider that was drawing 2° MaA fibres and both 1° and 2° MiA fibres (on both PMS). The MiA spigots of the four remaining spiders were not examined. Where 1° and/or 2° MiA fibres were observed on one PMS but not the other, we cannot rule out the possibility that fibres were severed while preparing the spinnerets for SEM.

In those spiders that were drawing 1° ampullate fibres, the ampullae and tails of the 1° ampullate glands were usually translucent, with plentiful accumulations of luminal material, and their ducts were crystalline in appearance and followed the indirect route typical of ampullate gland ducts. A slight departure from this description was observed in spiders killed shortly after ecdysis. Here, the 1° ampullate glands did not appear to be completely reformed, as mentioned above (see § 4*a*). However, sizable quantities of luminal material were present in these glands at this time and, overall, the course and appearance of their ducts were those of functional ampullate glands. It was, therefore, not surprising that fibres were observed exiting on the 1° ampullate spigots of spiders which had just completed ecdysis.

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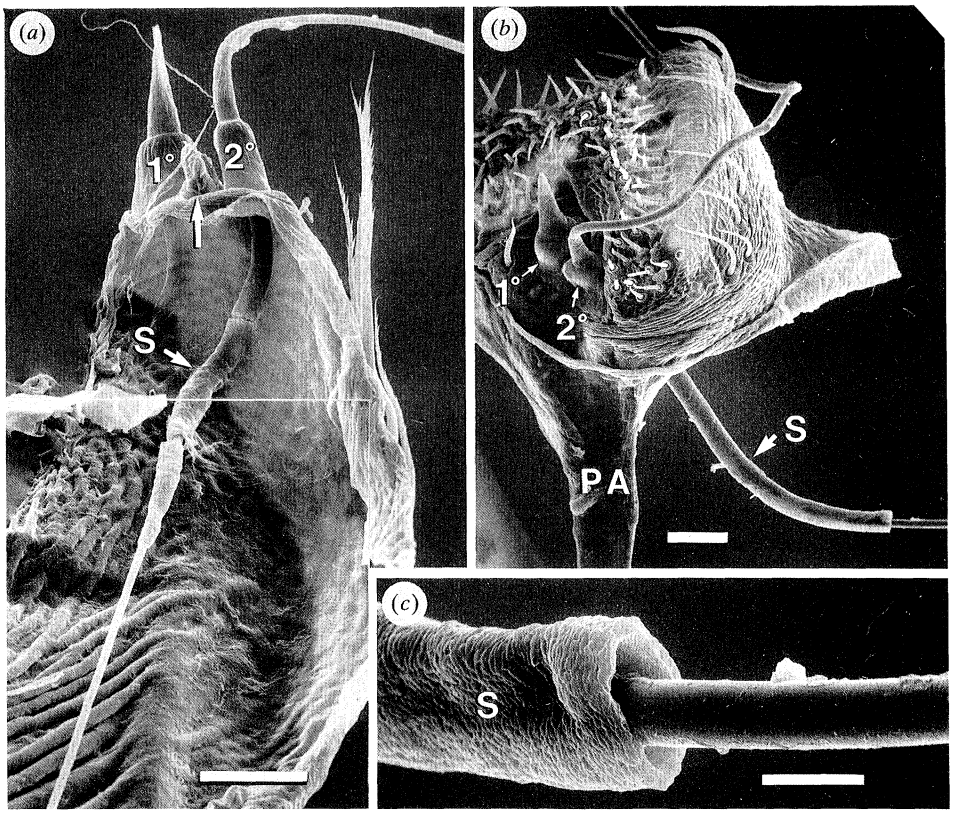

Figure 4. Portions of spinnerets from an exuvium shed by a ninth (penultimate) instar female *A. cavaticus*: (*a*) 1° and 2° MiA spigots on the right PMS, flanking a non-vestigial-type MiA nubbin (unlabeled arrow). (*b*) Distal segment of the left ALS showing the 1° and 2° MaA spigots, bordered on three sides by the pyriform spinning field. Silk fibres pass through the 2° ampullate spigots in both (*a*) and (*b*) and sheaths (S), which begin at the 2° ampullate spigots, surround a portion of these fibres on the 'internal' side of the exuvium. The sheaths may be duct segments (at least the cuticular lining) lost at ecdysis. (*c*) Higher magnification view of the 2° MaA fibre from (*b*) at its point of entry into the sheath (S). PA, principal apodeme. Bars: (*a*,*b*) = 50 μm; (*c*) = 10 μm.

Another deviation from the above description was seen in the individual which was using 1° and open 2° MaA and MiA glands concurrently (killed 21.6 days after the most recent ecdysis, figure 7). This spider was apparently just entering the white opaque phase, as indicated by the whitish appearance of the epithelium of the 1° ampullate glands' ampullae/tails. There was, however, still a moderate amount of transparent, gel-like luminal material present and the ducts of these glands appeared to be functional. (The 1° ampullate glands of the other spider that was using both 1° and open 2° MaA glands did not show any definite signs of having entered the white opaque phase. However, this spider's reclusive behaviour would indicate that entrance into the white opaque phase was imminent.)

Recent entrance into the white opaque phase was also evident in the 1° ampullate glands of the spider that was drawing 1° and 2° MiA fibres, but only 2° MaA fibres (killed 19.5 days after the most recent ecdysis, figure 7). In addition to the whitish epithelium of the ampullae/tails, the 1° MaA glands had ducts which appeared non-functional in their first segment (i.e. from their juncture with the ampulla to just before the first hairpin turn). This segment was 'non-crystalline', white and delicate, in contrast to the rest of the duct, which still retained the 'crystallinity' of a functional duct. The ducts of the 1° MiA glands had not yet reached this early point in the white opaque phase. Instead, they appeared functional over their entire length. Evidently, though the 1° MaA and MiA glands undergo the same changes during proecdysis, perfect synchrony does not exist between the two ampullate gland types (contrary to the impression that was, perhaps, given previously (Townley *et al.* 1991)). On the basis of the observations made on this single spider, we tentatively propose that 1° MaA glands become non-functional ahead of the 1° MiA glands during proecdysis.

The 1° ampullate glands of those spiders that were not drawing any 1° ampullate fibres were either in the white opaque phase or were emerging from this phase. Because 1° ampullate gland ducts of proecdysial spiders are connected to the 1° ampullate spigots on the new cuticle, these glands cannot function in the brief interval between emergence from the white opaque phase and ecdysis, despite a basically functional appearance.

Those spiders drawing 2° MaA fibres had open 2° ampullate glands that were well developed, with clearly functional ducts, translucent ampullae/tails

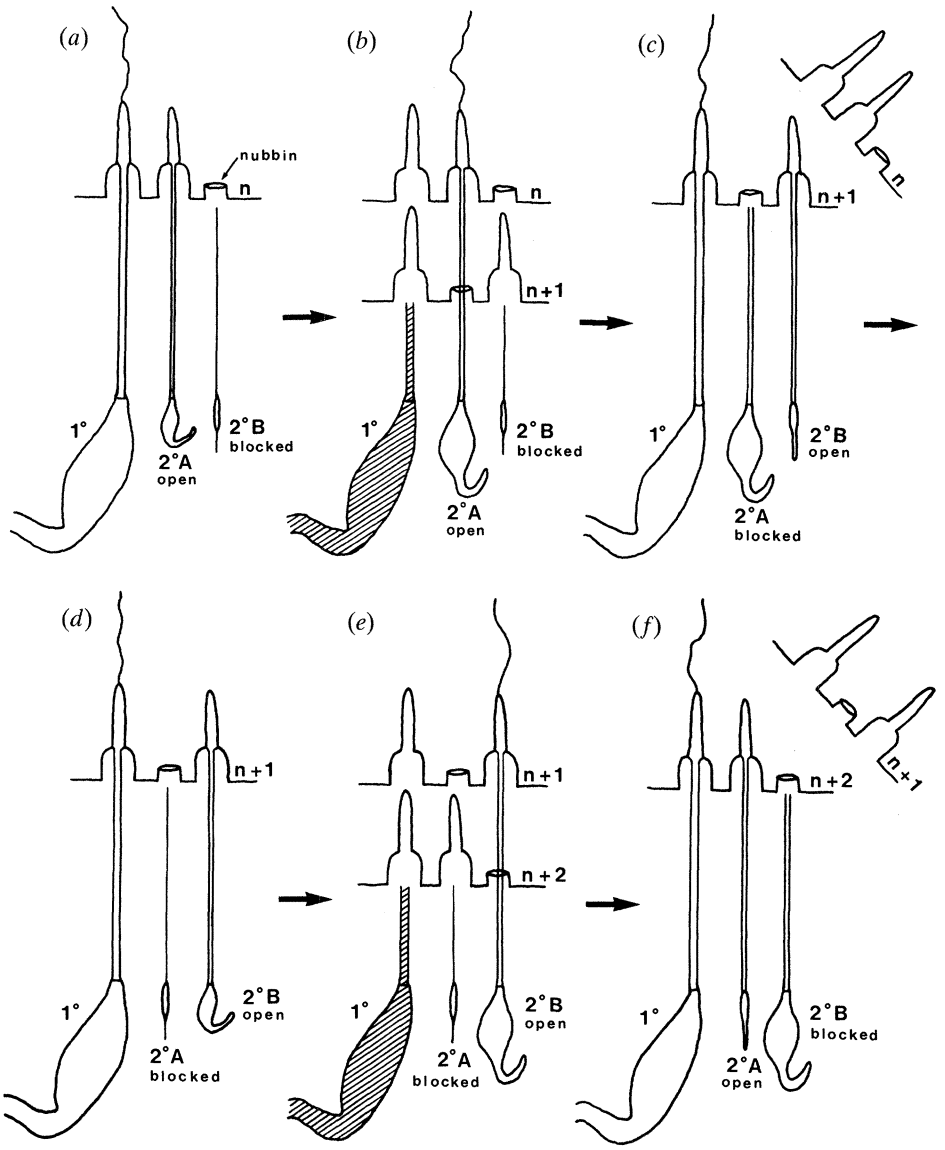

Figure 5. Diagrammatic summary of the principal cyclical changes that ampullate glands in *A. cavaticus* undergo during ontogeny. Note that only the ampullate glands associated with a single spinneret are depicted. During most of an interecdysial period the 1° ampullate glands are functional and are generally the only ampullate glands in use. Such a situation is presented in (*a*) at a point roughly midway in an interecdysial period. During proecdysis, however, the 1° ampullate glands enter a non-functional, white opaque phase (*b*), at which time the open 2° ampullate glands are functional and in use. Immediately after ecdysis (*c*), the 1° ampullate glands are again functional and the open and blocked 2° ampullate glands of the preceding stadium are blocked and open, respectively. The new blocked 2° ampullate glands regress during the first part of the interecdysial period while the open 2° ampullate glands enlarge, so that by about midway in the interecdysial period, the situation in (*d*) exists. Thus, the open 2° ampullate glands that are used during proecdysis (*e*) are the same glands that were blocked in the preceding stadium (*a*,*b*) and that again become blocked after ecdysis (*f*). These events occur in both MaA and MiA glands. However, 2° ampullate spigots and nubbins switch positions with each other, as shown here, only in the case of MiA glands.

and substantial accumulations of luminal material. The same description applies to the open 2° ampullate glands of those three individuals, mentioned above, that were using MaA glands of the 1° type only, yet were able to produce 2° MiA fibres. (These three spiders were examined 10.6 or more days after ecdysis.) Indeed, most spiders that were dissected about one week or more after ecdysis had open 2° ampullate glands which appeared to be functional. Nevertheless, if the 1° ampullate glands had not yet entered the white opaque phase, these seemingly functional open 2° ampullate glands were typically not found to be in use.

A summary of ampullate gland usage is presented in figure 8.

5. DISCUSSION

(*a*) *Internal anatomy: ampullate glands*

The dissections performed in an earlier study focused largely on the transition from the penultimate instar

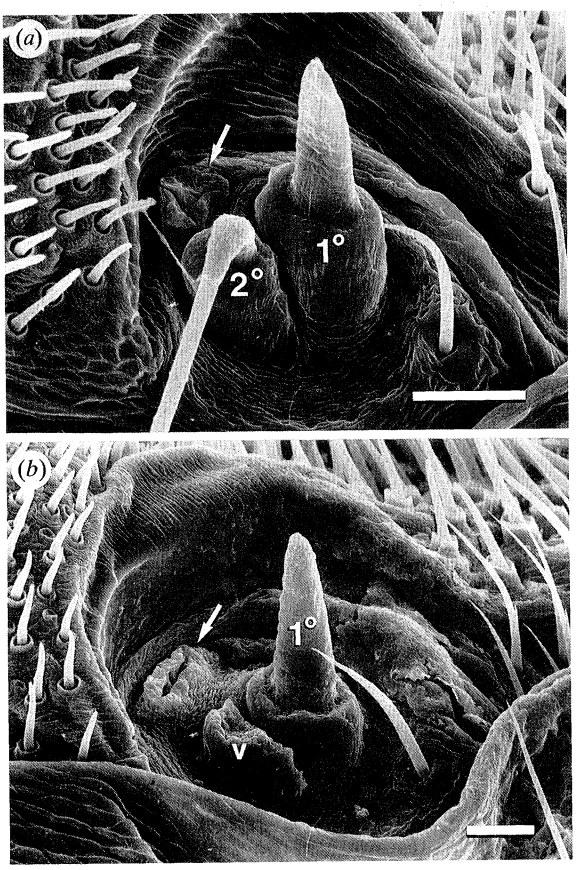

Figure 6. (*a*) 1° and 2° MaA spigots on the right ALS of a female *Argiope aurantia* in the penultimate stadium. (*b*) 1° MaA spigot and vestigial-type MaA nubbin (v) on the right ALS of an adult female *Argiope trifasciata*. An arrow in each photomicrograph points to the non-vestigial-type MaA nubbin (i.e. formed by the passage of an open 2° MaA duct through the cuticle during proecdysis, see Discussion). The fusules (upper right corner and along left side) in (*a*) and (*b*) serve pyriform glands. Several pyriform tartipores can be seen among the fusules. Bars = 25 μm.

to the adult (Townley *et al.* 1991). The only proecdysial spiders dissected in that study were penultimate instars. Thus, we did not know if 1° ampullate glands passed through a non-functional, white opaque phase in each juvenile stadium (in response to moulting cues) or if this phenomenon was unique to penultimates. Having now examined proecdysial third to penultimate instars, all evidence supports the former possibility. In contrast, the open 2° ampullate glands of these instars are functional during proecdysis. Apparently, for *A. cavaticus* (and no doubt other taxa as well), there is considerable survival value in being able to produce ampullate fibres at all times.

Dissections of prepenultimate juveniles, especially those performed shortly before and after ecdysis, have also clarified the true nature of 'accessory' MaA and MiA glands. Initially we assumed that the 'accessory' ampullate glands observed in a fifth instar, for example, were the same 'accessory' ampullate glands which were present in the fourth instar and which would have been present in the sixth instar; likewise for the 'smaller' MaA and MiA glands. We now recognize that the 'accessory' ampullate glands present throughout most of one stadium develop into 'smaller' ampullate glands during the next stadium and then revert back to 'accessory' ampullate glands in the stadium after that (figure 5). That is, the situation, with respect to 'accessory' and 'smaller' ampullate glands, only appeared static from one stadium to the next because there are two sets of (what we now call) 2° ampullate glands, which take turns functioning in successive stadia. (Each set consists of one pair of 2° MaA glands and one pair of 2° MiA glands.) This cycle of development and regression continues through all the juvenile stadia until, at the final moult, the two pairs of 'accessory' (i.e. blocked 2°) ampullate glands in proecdysial penultimates do not re-develop, but are simply retained in the adult in this regressed, non-functional form. Because the two pairs of 'smaller' (i.e. open 2°) ampullate glands in proecdysial penultimates atrophy in adults, a total of four pairs of 'accessory' (blocked 2°) ampullate glands are present in adults.

In part, we have adopted a new terminology for 'accessory' and 'smaller' ampullate glands because the 'accessory' ampullate glands present in a juvenile soon after ecdysis are not the same 'accessory' ampullate glands present later in the same interecdysial period; which is to say that the gross morphological features formerly used to identify 'accessory' ampullate glands are most apparent in the open 2° ampullate glands for a short time after ecdysis and in the blocked 2° ampullate glands subsequently. The same holds true for the 'smaller' ampullate glands, except, of course, it is the blocked 2° ampullate glands which have the appearance of 'smaller' ampullate glands immediately

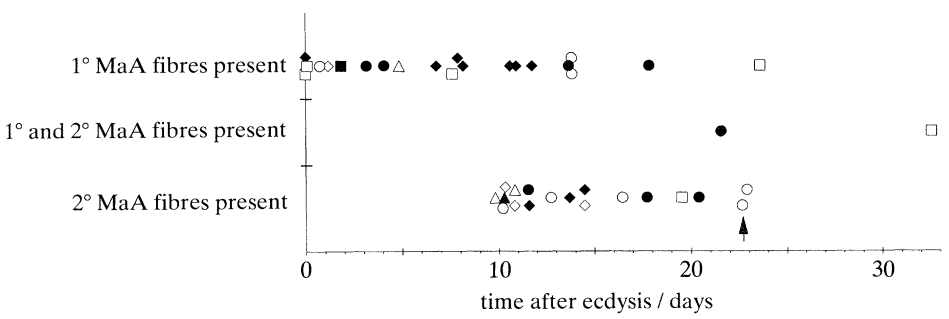

Figure 7. 1° and/or 2° MaA gland usage at known times after the most recent ecdysis. Each point represents one spider. Open symbols: males. Solid symbols: females. Triangles: sixth instars. Diamonds: seventh instars. Circles: eighth instars. Squares: ninth instars. Circle above arrow: tenth instar.

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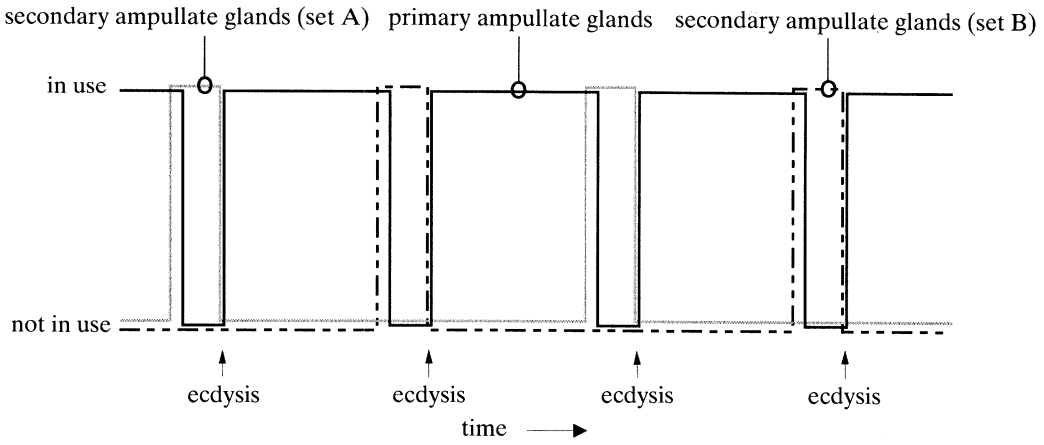

Figure 8. Generalized representation of ampullate gland usage by juvenile *A. cavaticus*. The relative lengths of time during which fibres are drawn from 1° and/or 2° ampullate glands, as depicted here, are only estimates. There is undoubtedly interindividual and interstadial variation in these ratios. Each set of 2° ampullate glands consists of one pair of 2° MaA glands and one pair of 2° MiA glands.

after ecdysis and the open 2° ampullate glands which do later in the interecdysial period. By differentiating these glands based on the connections their ducts make (or do not make) with spigots, attention is focused on the distinction between glands which will be or are functioning in a particular stadium and glands which will not or are not functioning; a distinction which remains clearly evident throughout an interecdysial period despite the dramatic morphological changes which these glands undergo.

(*b*) *External anatomy: nubbins and spigots*

The idea that there are two sets of 2° ampullate glands, and that one set functions in even-numbered juvenile instars while the other set functions in odd-numbered juvenile instars, arose (i) from the observation that 2° MiA spigots and MiA nubbins seemed to switch positions after each ecdysis (Townley *et al.* 1991), and (ii) from the presence of 'accessory' ampullate glands in juveniles (Atanasiu–Dumitresco 1941; Townley *et al.* 1991). However, because the former observation was made using spiders that were not raised in the laboratory, we could only estimate which stadia they were in. Thus, while the pattern that emerged was convincing, we could not be certain that there was a strict alternation, from stadium to stadium, in the positions occupied by 2° MiA spigots and MiA nubbins. In the present study, stadia were still only estimated, but in addition to examining the spiders themselves, one or more exuvia from these spiders were also examined. Without exception, 2° MiA spigots and MiA nubbins switched positions with each ecdysis in all individuals examined.

Previously, nubbins, including those observed in juveniles, have been interpreted as vestiges of spigots present in earlier stadia (Yu & Coddington 1990; Townley *et al.* 1991). It is now clear that, on the basis of origin, there are actually two types of nubbins, only one of which seems to represent vestigial spigots. Nubbins of the non-vestigial type are formed as a result of the passage of certain silk gland ducts through the cuticle while it is still forming (in the period between apolysis and ecdysis). In this way the silk glands served by these ducts are able to maintain their connections to spigots on the older, overlying cuticle and function during proecdysis, despite the intervening new cuticle. At the sites of duct penetration, collar-like protuberances form in the developing cuticle around the ducts. After ecdysis, these protuberances result in nubbins which often project above the surrounding cuticle. This type of nubbin is present in the cuticles of second instar through adult *A. cavaticus*. Vestigial-type nubbins, however, are only observed in adult *A. cavaticus* and seem to be aborted spigots which occupy the sites that would have been occupied by functional spigots, had the spider remained a juvenile.

In light of the above findings, the misconceptions in earlier papers are easily recognized. Thus, regarding the two MiA nubbins present on each PMS in adult *A. cavaticus*, we stated that 'Presumably, one of these nubbins is the typical remnant of the smaller MiA spigot and the other is the continued expression of the nubbin present in the penultimate . . .' (Townley *et al.* 1991). This misinterpretation resulted from the assumption that vestigial-type MiA nubbins occupy the sites occupied by 2° MiA spigots in penultimates. We now propose that the site occupied by a 2° MiA spigot in a penultimate is occupied by a non-vestigial-type MiA nubbin in the adult and that the other MiA nubbin on the PMS is of the vestigial type, being located where the 2° MiA spigot would have formed, were the spider not an adult. However, as 2° MaA spigots and non-vestigial-type MaA nubbins do not switch positions following an ecdysis, the vestigial-type MaA nubbins of adults are indeed located at the same sites occupied by 2° MaA spigots in penultimates. If our present interpretations are correct, the MiA and MaA arrangements demonstrate that vestigial-type and non-vestigial-type nubbins, respectively, are not necessarily located at the sites occupied by spigots in the preceding stadium.

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In figure 13 of Townley *et al.* (1991) the 1° and 2° MaA spigots on the old cuticle of one ALS from a proecdysial spider are shown along with a portion of the duct serving the 2° MaA spigot, not unlike the top half of figure 2*a* in the present paper. However, the former figure was intended to demonstrate the increased fragility of 1° ampullate gland ducts during the white opaque phase; the assumption being that a 1° MaA gland duct is absent in the preparation shown solely because of its inability to withstand the rigor of dissection. We are now aware that a 1° ampullate gland's connection to the old cuticle is severed in proecdysial spiders as a normal part of moulting.

The point was made previously (Townley *et al.* 1991) that functional ampullate glands would seem to be of no use in proecdysial *A. cavaticus* were the pyriform glands not also functional, as the latter produce the attachment disks which secure ampullate fibres to substrates (e.g. Apstein 1889; Warburton 1890; Peters 1955; Richter *et al.* 1971) and, very likely, the junctional cements which bind intersecting ampullate fibres to one another (Warburton 1890; Kavanagh & Tillinghast 1979; Work 1981; see also Jackson 1971). We have since observed pyriform gland ducts of proecdysial *A. cavaticus* penetrating the new cuticle and connecting to fusules on the old cuticle, very much in the manner of open 2° ampullate gland ducts. Thus, it seems probable that the tartipores present in the pyriform spinning fields of members of at least several araneomorph superfamilies (Kovoor 1986; Platnick 1990; Yu & Coddington 1990; M. A. Townley, E. K. Tillinghast & N. A. Cherim, personal observations; see also ALS figures in Coddington (1989); Peters & Kovoor (1991); Peters (1992)) are not vestigial fusules, but evidence of the prior passage of pyriform gland ducts through the cuticle. (Just as the terms fusule and spigot (fusule and cône, by French-speaking authors) have been applied by various authors (e.g. Wasowska 1970; Mikulska & Wiśniewski 1979; Kovoor 1986; Yu & Coddington 1990) to distinguish morphologically multiple and singular spigots, respectively (see Coddington 1989), so Yu & Coddington (1990) have used the terms tartipore and nubbin to distinguish morphologically multiple nubbins, such as those resulting from pyriform glands, from morphologically singular nubbins, such as those resulting from ampullate glands, respectively.) No attempt has yet been made to determine what changes the pyriform glands themselves undergo during ontogeny, but, like the open 2° ampullate glands, the pyriform glands functioning in proecdysial spiders cannot function after ecdysis, if only because their outlets (fusules) are removed with the old cuticle. Consequently, we presume there are additional pyriform glands in proecdysial spiders, the ducts of which are connected to fusules on the new cuticle. Following ecdysis these glands would be able to function.

We suspect the aciniform tartipores present on the posterior lateral spinnerets (PLS) of some araneoids (Yu & Coddington 1990; M. A. Townley, E. K. Tillinghast & N. A. Cherim, personal observations; see also figure 52*b* in Kovoor (1987)) are also non-vestigial-type nubbins, although we have not made direct observations to confirm this. (At this time we do not know if aciniform tartipores are present on the PMS of *A. cavaticus*.) Aciniform tartipores, which presumably arise by the same mechanism, can also be seen on the PMS and PLS of the deinopid *Deinopis subrufa* (figures 4*c*, 5*a–c*, 6*b* in Peters (1992)). Indeed, tartipores are apparently present on the PMS and/or PLS of at least some mygalomorph spiders (Glatz 1973; Shear *et al.* 1989; see also figure 52*a* in Kovoor (1987) and the figures presented in the survey by Palmer (1990), particularly figures 9*h*, 10*f* and 14*d*). The aggregate and flagelliform nubbins of adult male araneoids (Sekiguchi 1955*a*,*b*), however, are additional examples of the vestigial type of nubbin (see figure 3*b* in Peters & Kovoor (1991); figure 16 in Townley *et al.* (1991)), as are the pseudoflagelliform and paracribellar nubbins of adult male deinopoids (Kovoor & Peters 1988; Peters 1992).

Though difficult to observe in *A. cavaticus*, non-vestigial-type MaA nubbins, one per ALS, are conspicuous in some other araneids (figure 6, this paper; figure 21 in Yu & Coddington (1990); figures 15 and 19 in Coddington (1989)). They occupy sites lateral or posterolateral to either vestigial-type MaA nubbins or 2° MaA spigots, depending on whether the spider is an adult or a juvenile. They also appear to be present, in the same locations, in at least some non-araneid araneoids, including *Latrodectus* species (Theridiidae) (figure 27 in Coddington (1989); M. A. Townley, E. K. Tillinghast & N. A. Cherim, personal observations), *Dipoena beni* (Theridiidae) (figure 344 in Forster *et al.* (1990)) and *Linyphia triangularis* (Linyphiidae) (figure 2*b* in Peters & Kovoor (1991)), as well as in the uloborid *Octonoba octonarius* (figure 7 in Coddington (1989)). (They are unlabeled in the figures cited.) However, the evidence to date (Coddington 1989; Forster *et al.* 1990) suggests that theridiids and most linyphiids lack functional 2° MiA glands both as juveniles and adults. Thus, while these taxa apparently employ open 2° MaA glands during proecdysis, the use of MiA glands is presumably forgone altogether during this period.

In adult *Deinopis*, multiple MaA spigots and nubbins are present on each ALS (Coddington 1989; Peters 1992). Earlier, we assumed these (and all) ampullate nubbins to be vestigial and, thus, concluded that there was a reduction in the number of functional MaA glands, in going from the penultimate stadium to the adult (Townley *et al.* 1991). However, the appearance of these nubbins in figure 2*b* of Peters (1992) suggests non-vestigial-type nubbins. Therefore, a reduction in the number of MaA glands is actually not indicated.

(*c*) *Ampullate gland usage*

In figure 7 the reader will note that some spiders continued to use 1° MaA glands beyond the time at which other spiders (in the same stadium and of the same gender) had switched over to 2° MaA glands. Presumably, this is a reflection of the variation which exists in the length of particular interecdysial periods; variation which was undoubtedly increased by the

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different feeding rates among spiders. Variation may be further increased among eighth instars at least because of the variable number of stadia that *A. cavaticus* can potentially pass through before becoming adults. Some spiders are in the penultimate stadium when they are eighth instars, others when they are ninth, or possibly even tenth, instars. (The possibility that some *A. cavaticus* reach the final juvenile stadium when they are tenth instars was raised previously (Townley *et al.* 1991). Although infrequent, we again encountered penultimates in the present study that had carapace dimensions and PMS aciniform fusule complements such as to indicate that they were tenth instars. One such individual has been designated a tenth instar in figure 7. However, this designation must be viewed as tentative.) Despite this variation, the data demonstrate that 1° ampullate glands are used exclusively during the first portion of an interecdysial period and that 1° and 2° ampullate glands are typically not used concurrently. Further, when these data are coupled with observations made on the ampullate glands of the same individuals, several trends in ampullate gland usage are indicated.

First, if the 1° MaA glands are functional, they are being used (not continuously, of course). Thus, in our study, if the 1° ampullate glands appeared to be functional (i.e. if they had substantial accumulations of clear, gel-like luminal material in their ampullae and zig-zagging ducts which were crystalline in appearance and 'open'), then at least 1° MaA fibres were observed upon examination by SEM. (Not unexpectedly, the use of MiA glands, 1° and 2°, was more variable. In structures formed principally from MaA fibres, such as draglines and various orb web elements, the occurrence of MiA fibres is also variable (Kavanagh & Tillinghast 1979; Work 1981). Of considerable interest, therefore, is the discovery of structures containing MiA, but not MaA, fibres; namely, the bridging lines produced by *Araneus diadematus* and *Linyphia triangularis* (Peters 1990).)

Second, if the 1° ampullate glands are functional, the 2° MaA glands are typically not used, even if they are also functional. Again, this conclusion is based on appearances of dissected glands and one cannot be certain that functional-looking glands actually are functional. However, as mentioned earlier, three spiders that were drawing only 1° MaA fibres were also drawing, or could have drawn from them, single 2° MiA fibres. Taken together, these observations suggest that 2° ampullate glands become functional before the 1° ampullate glands enter the white opaque phase, but they are generally not used until that point is reached. In three instances the concurrent use of 1° and 2° MaA and/or MiA glands was observed in spiders that were entering or close to entering the white opaque phase. Thus, a transitional period can apparently occur shortly before the 1° ampullate glands become non-functional. Exclusive use of the 2° ampullate glands seemingly occurs only once the 1° ampullate glands actually become incapable of functioning.

Third, 2° ampullate glands are not generally, if at all, used in the building of orb webs. Like the 1° ampullate glands, the flagelliform and aggregate glands are apparently non-functional during much of proecdysis, and since the latter two gland types produce the core fibres and viscid cover, respectively, of the adhesive spiral (Sekiguchi 1952; Peters 1955), orb web construction is not possible at this time. It is at this very time, however, that the 2° ampullate glands are in use. Of course, if 2° ampullate glands are functional at least briefly before the flagelliform and aggregate glands become non-functional, then there is the possibility that 2° ampullate fibres are incorporated into orb webs built in the latter portion of the interecdysial period. After all, though our observations indicate that 2° ampullate fibres are not generally used to produce draglines until the start of the white opaque phase, we do not know that the spider similarly refrains from using 2° ampullate fibres in producing orb web components, if such is possible. However, considering just those spiders that were found producing 2° MaA and/or 2° MiA fibres, the shortest interval between the construction of the most recent orb web and preparation of the spinnerets for SEM was about 32 hr, and that was for a spider that was apparently just entering the white opaque phase (and was producing 1° and 2° MaA and MiA fibres). Thus, to date, we have no evidence which indicates that 2° ampullate fibres are used in building orb webs. The question of 2° ampullate fibre use in orb webs could be profitably explored using Peters' (1982) paraffin technique (unmodified).

We thank Dr Donald Chandler, UNH Department of Entomology, for identification of flies, as well as Veronica and Gene Specyalski for their generous provision of spiders. This study was supported in part by NIH grant 1-R15-GM44353-O1A1 and funds from the Agricultural Experiment Station (HATCH 351).

REFERENCES

Apstein, C. 1889 Bau und Function der Spinndrüsen der Araneida. *Arch. Naturgesch.* **55**, 29–74.

Atanasiu–Dumitresco, M. 1941 Contributions a l'étude anatomique et cytologique de l'appareil séricigène des araignées. *Anal. Acad. Rom. Mem. Sect. Stiint., Ser. 3* **16**, 773–840.

Bell, A.L. & Peakall, D.B. 1969 Changes in fine structure during silk protein production in the ampullate gland of the spider *Araneus sericatus*. *J. Cell Biol.* **42**, 284–295.

Coddington, J.A. 1989 Spinneret silk spigot morphology: evidence for the monophyly of orbweaving spiders, Cyrtophorinae (Araneidae), and the group Theridiidae plus Nesticidae. *J. Arachnol.* **17**, 71–95.

Forster, R.R., Platnick, N.I. & Coddington, J. 1990 A proposal and review of the spider family Synotaxidae (Araneae, Araneoidea), with notes on theridiid interrelationships. *Bull. Am. Mus. nat. Hist.* **193**.

Glatz, L. 1973 Der Spinnapparat der Orthognatha (Arachnida, Araneae). *Z. Morph. Tiere* **75**, 1–50.

Groome, J.R., Townley, M.A., de Tschaschell, M. & Tillinghast, E.K. 1991 Detection and isolation of proctolin-like immunoreactivity in arachnids: possible cardioregulatory role for proctolin in the orb-weaving spiders *Argiope* and *Araneus*. *J. Insect Physiol.* **37**, 9–19.

Jackson, R.R. 1971 Fine structure of thread connections in the orb web of *Araneus diadematus*. *Psyche* **78**, 12–31.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Johansson, B. 1914 Zur Kenntnis der Spinndrüsen der Araneina. *Acta Univ. Lund., N.S.* **10**(5), 1–12.

Kavanagh, E.J. & Tillinghast, E.K. 1979 Fibrous and adhesive components of the orb webs of *Araneus trifolium* and *Argiope trifasciata*. *J. Morph.* **160**, 17–31.

Kovoor, J. 1977 Données histochimiques sur les glandes sericigènes de la veuve noire *Latrodectus mactans* Fabr. (Araneae, Theridiidae). *Ann. Sci. Nat. Zool. Biol. Anim. Sér. 12* **19**, 63–87.

Kovoor, J. 1979 Les glandes sericigènes d'*Uroctea durandi* (Latreille) (Araneae: Oecobiidae). Révision, histochimie, affinités. *Ann. Sci. Nat. Zool. Biol. Anim. Sér. 13* **1**, 187–203.

Kovoor, J. 1986 L'appareil sericigène dans les genres *Nephila* Leach et *Nephilengys* Koch: anatomie microscopique, histochimie, affinités avec d'autres Araneidae. *Rev. Arachnol.* **7**, 15–34.

Kovoor, J. 1987 Comparative structure and histochemistry of silk-producing organs in arachnids. In *Ecophysiology of spiders* (ed. W. Nentwig), pp. 160–186. Berlin: Springer-Verlag.

Kovoor, J. & Peters, H.M. 1988 The spinning apparatus of *Polenecia producta* (Araneae, Uloboridae): structure and histochemistry. *Zoomorphology* **108**, 47–59.

Kovoor, J. & Zylberberg, L. 1972 Morphologie et ultrastructure du canal des glandes ampullacées d'*Araneus diadematus* Clerck (Arachnida, Araneidae). *Z. Zellforsch. mikrosk. Anat.* **128**, 188–211.

Meckel, H. 1846 Mikrographie einiger Drüsenapparate der niederen Thiere. *Arch. Anat. Physiol. wiss. Med. (Müller's Arch.)* 1–73.

Mikulska, I. & Wiśniewski, H. 1979 Variation in the number of external spinning structures in the females of *Cyphepeira patagiata* (Clerck) (Araneae, Argiopidae) in population of Peninsula Hel. *Zool. Polon.* **27**, 179–186.

Moon, M.J. & Kim, W.K. 1989 Ultrastructure of the ampullate glands in the orb web spider, *Nephila clavata* L. Koch. III. Excretory duct of the small ampullate gland. *Korean J. Electron Microsc.* **19**, 49–58.

Moon, M.J., Kim, C.S. & Kim, W.K. 1988 Ultrastructure of the ampullate gland in the orb web spider, *Nephila clavata* L. Koch. I. Excretory duct of the large ampullate gland. *Korean J. Electron Microsc.* **18**, 77–90.

Mullen, G.R. 1969 Morphology and histology of the silk glands in *Araneus sericatus* Cl. *Trans. Am. Microsc. Soc.* **88**, 232–240.

Palmer, J.M. 1990 Comparative morphology of the external silk production apparatus of 'primitive' spiders. Ph.D. thesis, Harvard University.

Peters, H.M. 1955 Über den Spinnapparat von *Nephila madagascariensis* (Radnetzspinnen, Fam. Argiopidae). *Z. Naturforsch.* **10b**, 395–404.

Peters, H.M. 1982 Wie Spinnen der Familie Uloboridae ihre Beute einspinnen und verzehren. *Verh. naturwiss. Ver. Hamburg (NF)* **25**, 147–167.

Peters, H.M. 1990 On the structure and glandular origin of bridging lines used by spiders for moving to distant places. *Acta Zool. Fennica* **190**, 309–314.

Peters, H.M. 1992 On the spinning apparatus and the structure of the capture threads of *Deinopis subrufus* (Araneae, Deinopidae). *Zoomorphology* **112**, 27–37.

Peters, H.M. & Kovoor, J. 1991 The silk-producing system of *Linyphia triangularis* (Araneae, Linyphiidae) and some comparisons with Araneidae. *Zoomorphology* **111**, 1–17.

Platnick, N.I. 1990 Spinneret morphology and the phylogeny of ground spiders (Araneae, Gnaphosoidea). *Am. Mus. Novitates* No. **2978**.

Richter, C.J.J., Stolting, H.C.J. & Vlijm, L. 1971 Silk production in adult females of the wolf spider *Pardosa amentata* (Lycosidae, Araneae). *J. Zool., Lond.* **165**, 285–290.

Sekiguchi, K. 1952 On a new spinning gland found in geometric spiders and its functions. *Annot. Zool. Japon.* **25**, 394–399.

Sekiguchi, K. 1955*a* Differences in the spinning organs between male and female adult spiders. *Sci. Rep. Tokyo Kyoiku Daigaku* B **8**, 23–32.

Sekiguchi, K. 1955*b* The spinning organs in sub-adult geometric spiders and their changes accompanying the last moulting. *Sci. Rep. Tokyo Kyoiku Daigaku* B **8**, 33–40.

Shear, W.A., Palmer, J.M., Coddington, J.A. & Bonamo, P.M. 1989 A Devonian spinneret: Early evidence of spiders and silk use. *Science, Wash.* **246**, 479–481.

Tillinghast, E.K. & Kavanagh, E.J. 1977 The alkaline proteases of *Argiope* and their possible role in web digestion. *J. exp. Zool.* **202**, 213–222.

Townley, M.A., Horner, N.V., Cherim, N.A., Tugmon, C.R. & Tillinghast, E.K. 1991 Selected aspects of spinning apparatus development in *Araneus cavaticus* (Araneae, Araneidae). *J. Morph.* **208**, 175–191.

Warburton, C. 1890 The spinning apparatus of geometric spiders. *Q. Jl microsc. Sci., N. S.* **31**, 29–39.

Wasowska, S. 1970 Structures fileuses extérieures sur les filières (thelae) de l'araignée *Argiope bruennichi* (Scopoli). *Zool. Polon.* **20**, 257–268.

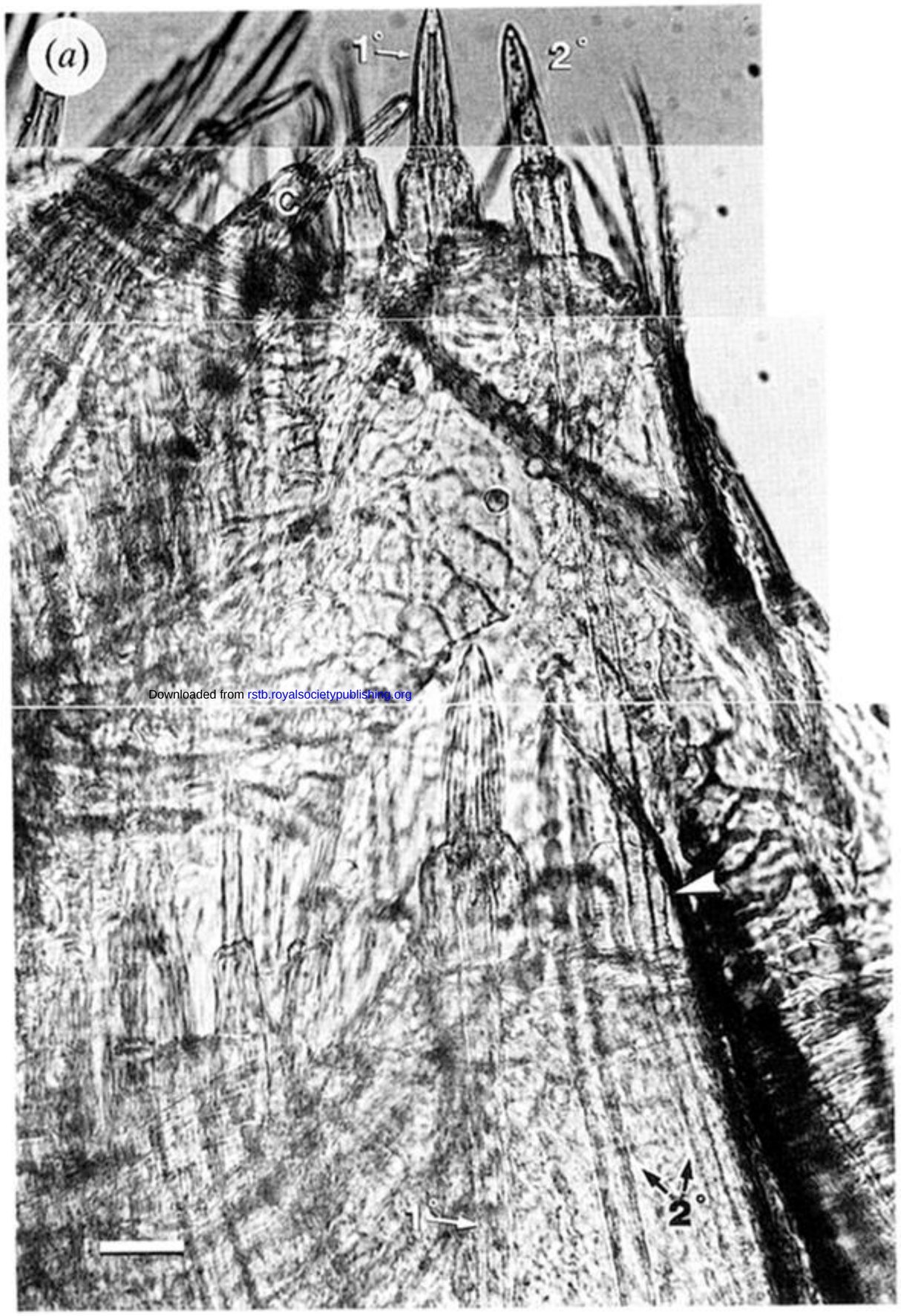
Wilson, R.S. 1969 Control of drag-line spinning in certain spiders. *Am. Zool.* **9**, 103–111.

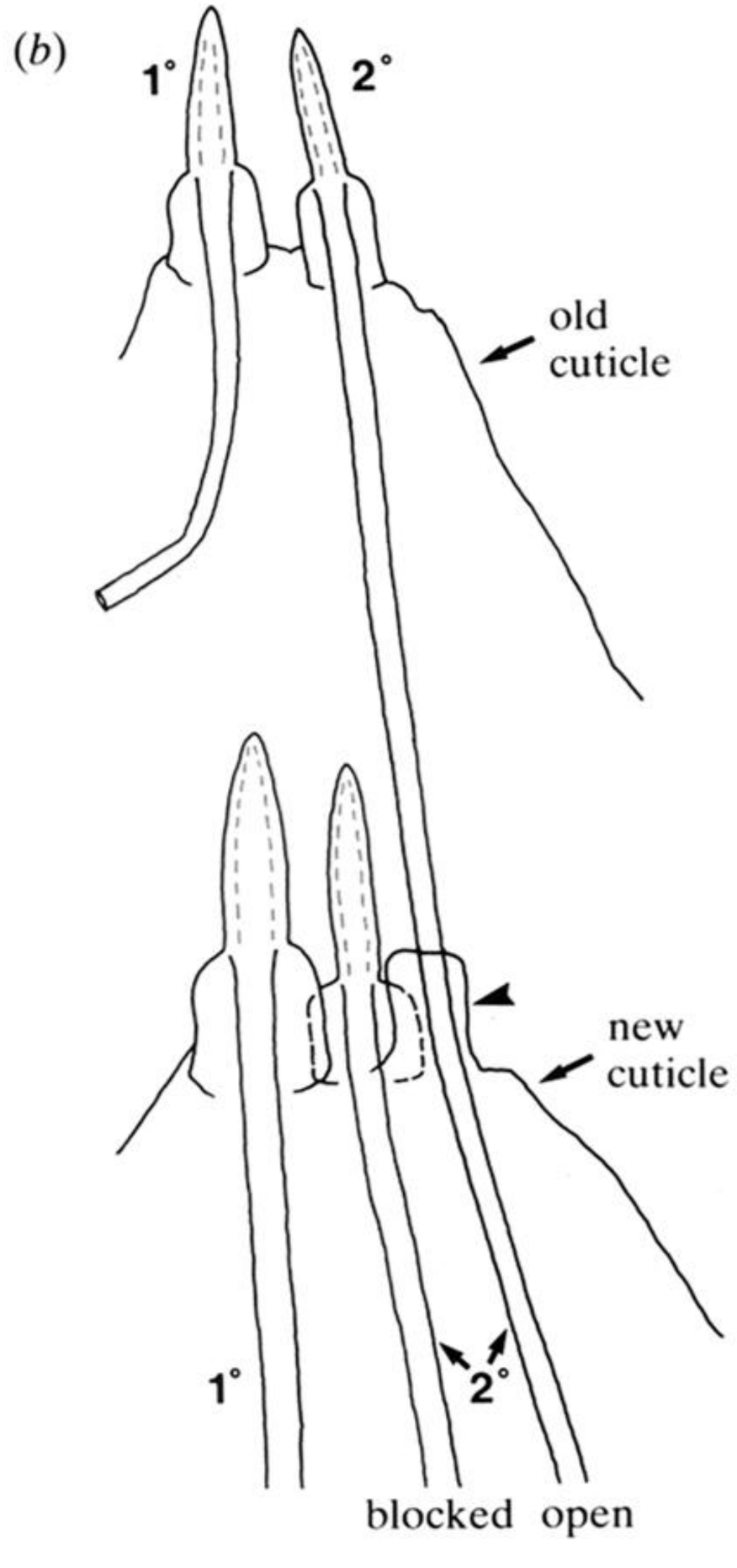
Work, R.W. 1981 Web components associated with the major ampullate silk fibers of orb-web-building spiders. *Trans. Am. microsc. Soc.* **100**, 1–20.

Yu, L. & Coddington, J.A. 1990 Ontogenetic changes in the spinning fields of *Nuctenea cornuta* and *Neoscona theisi* (Araneae, Araneidae). *J. Arachnol.* **18**, 331–345.

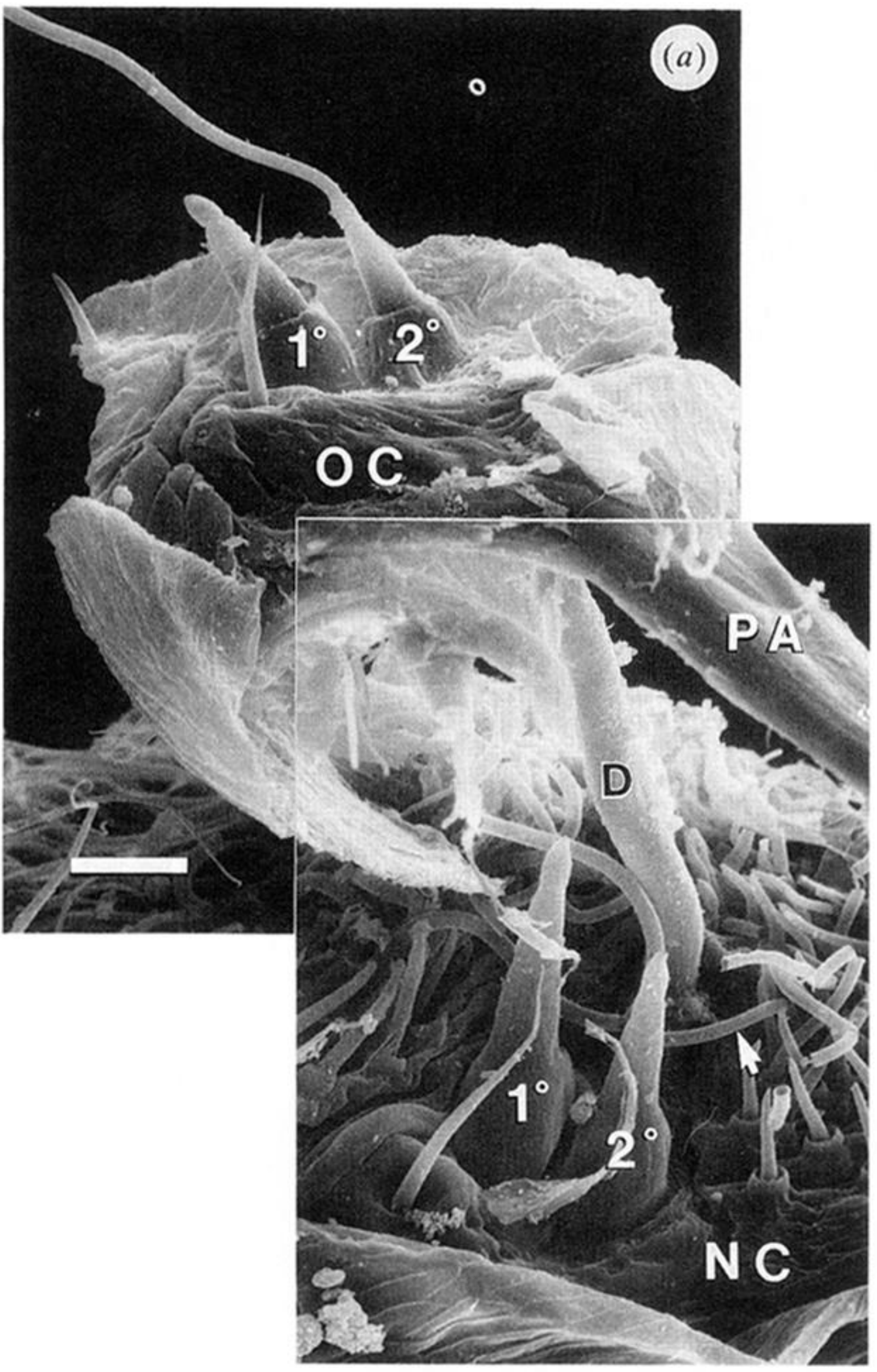
Received 30 July 1992; accepted 15 October 1992

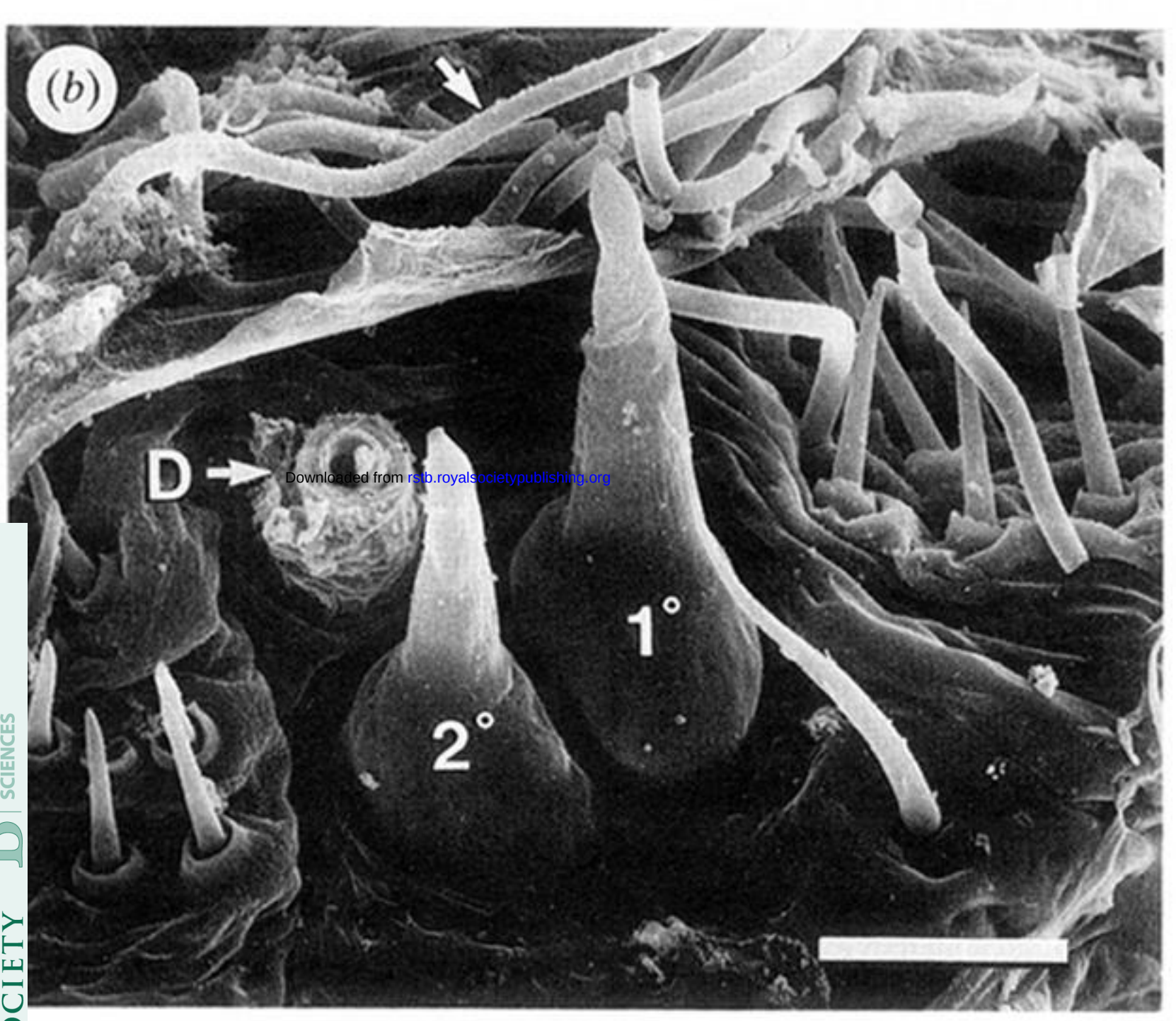
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



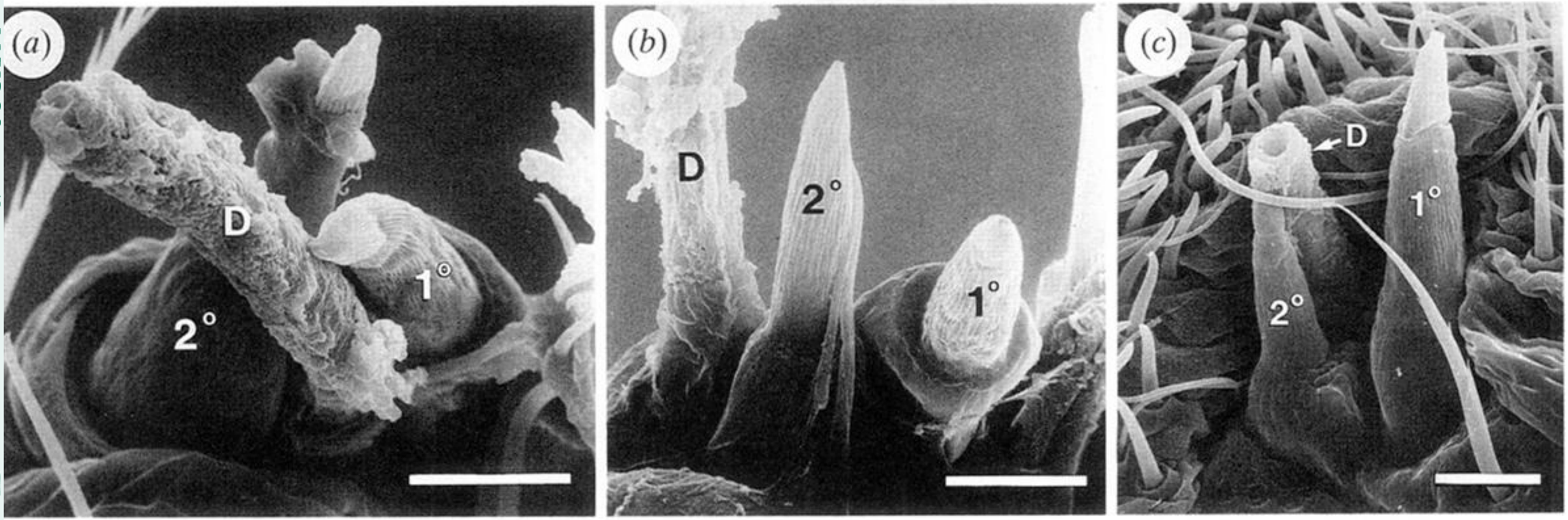


igure 1. (*a*) A portion of the left PMS from a seventh instar female *A. cavaticus*, close to ecdysing, showing the new uticle beneath the old cuticle. The diagram in (*b*) outlines those structures in the photomicrograph relevant to the pic at hand. Emergence from the white opaque phase was well under way by this time, so that the largely re-rmed duct of a 1° MiA gland is clearly visible connecting to the more anterior MiA spigot on the new cuticle. Only nce the old cuticle was removed at ecdysis would this gland have been able to resume functioning. A portion of uct is faintly visible connecting to the 1° MiA spigot on the old cuticle; a remnant, presumably, of the duct which as functioning prior to the white opaque phase. The partially re-formed duct of a blocked 2° MiA gland can be en attached to the more posterior MiA spigot on the new cuticle. This gland, very thin at this time,would not have en used until late in the following stadium. Connected to the 2° MiA spigot on the old cuticle and passing through e new cuticle is the duct of a well-developed, open 2° MiA gland. This gland would have remained functional ntil ecdysis. Note that the duct passes through a protuberance (nubbin progenitor) in the new cuticle (arrowheads (*a*) and (*b*)). The unidentified spinning structures to the left of the MiA spigots in (*a*) are aciniform fusules. C, ylindrical gland spigot. Bar = 25 μm.





gure 2. Seventh instar male *A. cavaticus* in proecdysis: (*a*) art of the left ALS showing the 1° and 2° MaA spigots on oth the old cuticle (OC) and the underlying, new cuticle NC). The duct (D) of an open 2° MaA gland can be seen assing through the new cuticle to maintain its attachment the 2° MaA spigot on the old cuticle, thus allowing the ider to continue drawing MaA fibres during proecdysis.) Part of the right ALS from the same individual, showing, ore precisely, the site at which open 2° MaA gland ducts enetrate the new cuticle in proecdysial spiders. Only the ew cuticle is shown. The duct (D) of the open 2° MaA and has been broken off just distal to the new cuticle. In) and (*b*) tubules can be seen scattered on the surface of e new cuticle (unlabeled arrows point to examples). These bules are portions of pyriform gland ducts which, like the pen 2° MaA gland ducts, were passing through the new ticle to connect to fusules on the old cuticle (and were vered during dissection). PA, principal apodeme (see Vilson 1969). Bars = 25 μm.



gure 3. 1° and 2° MiA (*a*,*b*) and MaA (*c*) spigots on the new (developing) cuticle of proecdysial *A. cavaticus*. (*a*) eft PMS from an eighth instar male, (*b*) left PMS from a seventh instar male, (*c*) right ALS from a sixth instar male. The overlying, old cuticle has been removed in these preparations (and the ducts of the open 2° ampullate ands severed from the old cuticle). In even-numbered instars (*a*) the ducts of the open 2° MiA glands pass through e new cuticle anterolateral to the 2° MiA spigots whereas in odd-numbered instars (*b*) the ducts pass osteromedial to the 2° MiA spigots. Open 2° MaA ducts penetrate the new cuticle lateral to the 2° MaA spigots both even-numbered (*c*) and odd-numbered instars (see figure 2*b*). D, duct of open 2° ampullate gland. ars = 25 μm.

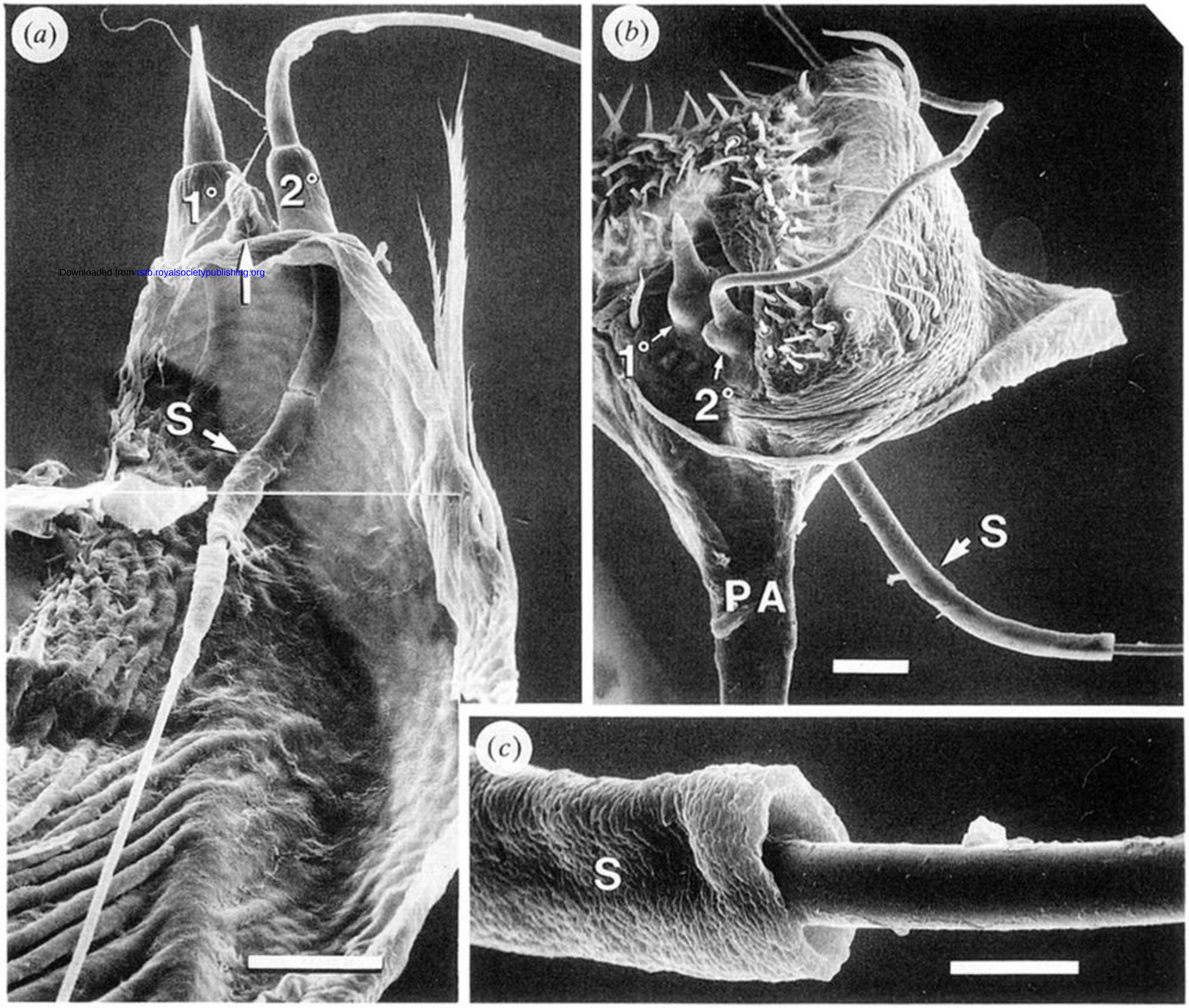
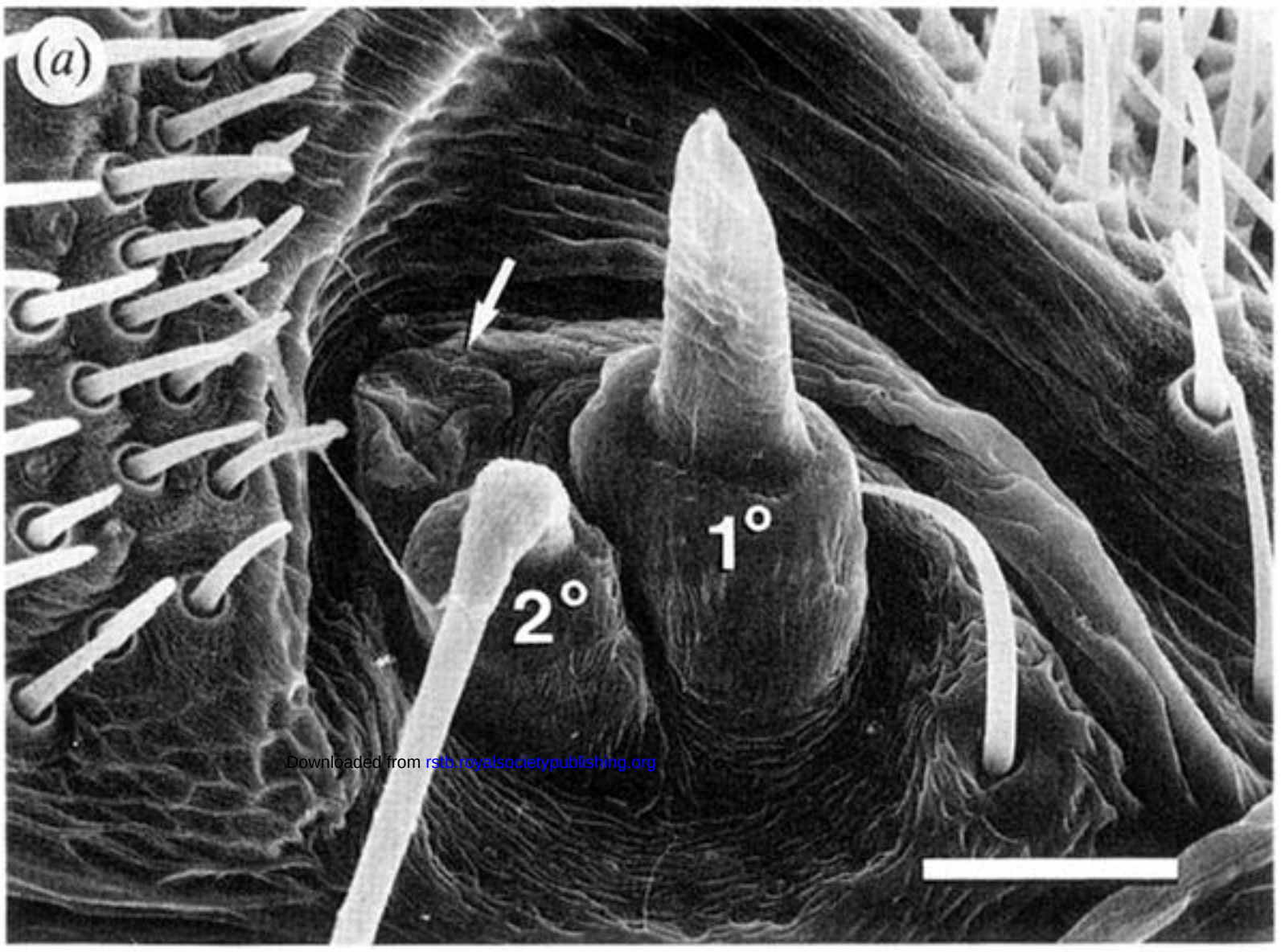
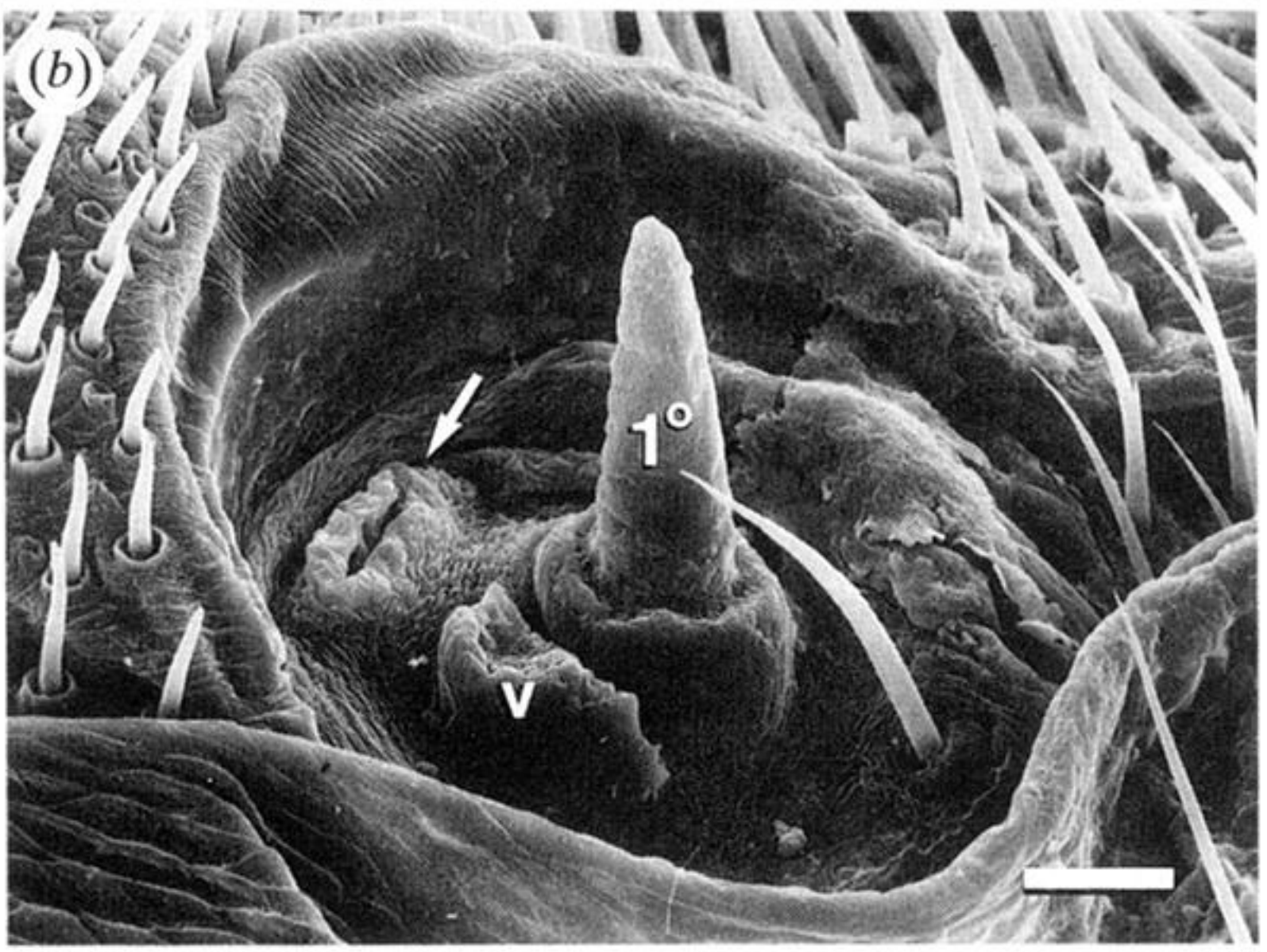


Figure 4. Portions of spinnerets from an exuvium shed by a ninth (penultimate) instar female *A. cavaticus*: (*a*) 1° and 2° MiA spigots on the right PMS, flanking a non-vestigial-type MiA nubbin (unlabeled arrow). (*b*) Distal segment of the left ALS showing the 1° and 2° MaA spigots, bordered on three sides by the pyriform spinning field. Silk fibres pass through the 2° ampullate spigots in both (*a*) and (*b*) and sheaths (S), which begin at the 2° ampullate spigots, surround a portion of these fibres on the 'internal' side of the exuvium. The sheaths may be duct segments (at least the cuticular lining) lost at ecdysis. (*c*) Higher magnification view of the 2° MaA fibre from (*b*) at its point of entry to the sheath (S). PA, principal apodeme. Bars: (*a*,*b*) = 50 μm; (*c*) = 10 μm.





gure 6. (*a*) 1° and 2° MaA spigots on the right ALS of a male *Argiope aurantia* in the penultimate stadium. (*b*) 1° aA spigot and vestigial-type MaA nubbin (v) on the right LS of an adult female *Argiope trifasciata*. An arrow in each notomicrograph points to the non-vestigial-type MaA ubbin (i.e. formed by the passage of an open 2° MaA duct rough the cuticle during proecdysis, see Discussion). The sules (upper right corner and along left side) in (*a*) and (*b*) rve pyriform glands. Several pyriform tartipores can be en among the fusules. Bars = 25 μm.