

An Adhesive Collophore May Help Direct the Springtail Jump

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ABSTRACT The collophore of *Entomobrya multifasciata* (Tullberg) (Collembola: Entomobryidae) is composed of four segments. The third segment telescopes in and out of the second and the fourth is an eversible vesicle that is entirely enclosed in the third when not deployed. Low temperature scanning electron micrographs document that the collophore may remain adhered to the substrate even as the springtail initiates its jump. This adherence appears to force the posterior abdomen into the air, causing the jump itself to be initiated in a forward direction and accompanied by a forward flip. Among the several other documented functions of the collophore, we suggest that it may serve to affect the direction and trajectory of the springtail during its jump.

KEY WORDS Collembola, low temperature scanning electron microscopy (LT-SEM), ventral tube, furcula, *Entomobrya multifasciata*

The collophore, a ventral appendage of the first abdominal segment, is an apomorphy of the Collembola (Lubbock 1873, Snodgrass 1993). It is a fusion of modified ventral appendages (Imms 1906, D'Haese 2004, Konopova and Akam 2014), bilobed, carrying two distal eversible vesicles that may in turn carry mechano- and chemoreceptors and osmotic transport cells (Eisenbeis 1974, Verhoef et al. 1979).

Despite its universal presence and hence apparent importance to the biology of Collembola, and despite the fact the Collembola are one the most abundant components of soil fauna (Ponge et al. 1997), the actual function of the collophore is poorly known. Several functions have been proposed, including osmoregulation (Eisenbeis 1974, Eisenbeis and Wichard 1975) and in the mechanics of jumping on the surface of water (Baudoin 1950), but many entomologists will recall the mystery of the collophore as taught in their university courses.

Adhesive qualities have also long been recognized (De Geer 1783, Imms 1906). Indeed, the scientific names of the springtail class and their specialized appendage are derived from ancient Greek words describing apparent adhesive characteristics: colle, phore, and embolon, for glue, carrier, and piston, respectively. Willem and Sabbe (1897) suggested the glue was secreted by the collembolan's cervical glands. In at least some species of the order of globular springtails, Symphyleona, the very long and more or less prehensile vesicles are coated with an adhesive substance that

permits the collembolan to right itself when on its back (Lubbock 1873, Brackenbury 1990, Zettel et al. 2000). This behavior was not observed in Entomobryomorpha and Poduromorpha, however; the eversible vesicles of these latter springtails are much shorter and in any case are not needed for these cylindrical springtails to right themselves (Brackenbury 1990).

Despite the long-ago hypothesized adhesive function of the collophore, recent consensus has de-emphasized it: an oft-used entomology textbook dismisses adhesion as a collophore function (Johnson and Triplehorn 2004). Using low-temperature scanning electron microscopy (LT-SEM), we present evidence that the springtail's collophore does serve an adhesive function. We also suggest that this adhesion may contribute to the mechanics of the springtail's jump.

Materials and Methods

The technique for preparing the Collembola for LT-SEM was described by Achor et al. (2001). Briefly, living *Entomobrya multifasciata* (Tullberg 1871) were placed on copper plates in a 30-cm round plastic arena. Liquid nitrogen was poured into the chamber, but not over the specimens, to freeze the Collembola instantaneously. The copper plates were removed from the plastic arena and stored in copper tubes in a liquid nitrogen Dewar until the specimens could be observed at a later date. A total of 300–400 Collembola was thus cryo-prepared over the course of several sessions. The LT-SEM observations were made by transferring the samples to a pre-chamber of a CT-1500HF Oxford Cryo-trans System (Oxford Instruments, Inc., Concord, MA). They were then freeze etched, sputter coated with 10nm of platinum, and transferred to the pre-cooled cryostage in the microscope. Samples were viewed at 2 kV accelerating voltage on a Hitachi S4100 (Pleasanton, CA) field emission scanning electron microscope. Stereo micrographs, with tilt angles 6° apart,

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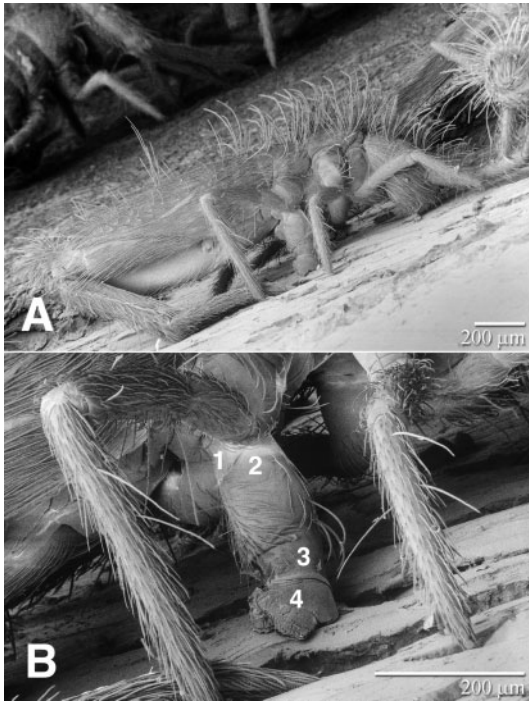


Fig. 1. Springtail at rest with collophore fully extended. (A) Habitus. (B) Close-up of collophore with segments 1–4 labeled.

were taken at a range of magnifications: 2.5 x–900 x. In most cases, only one of each pair of images is presented here.

Results

The collophore appears to be composed of four segments (Fig. 1). The first is short and triangular and may serve as the pivot against the ventral surface of the abdomen. The second is longer and, comparing Figs. 1 and 2, evidently acts as a sheath in and out of which the third segment telescopes. The third segment carries two distinct kinds of setae, approximately 10 filiform and 36 serrate setae, that presumably serve a sensory function (Fig. 2). Longer, thicker, serrate setae also extend from the anterior second segment and beyond the retracted third (Figs. 1 and 2). The fourth segment is the eversible vesicle. The vesicle is enclosed entirely within the third segment when the collophore is not adhered to the substrate (Fig. 2), but it is visibly everted when it is in contact (Fig. 1).

Several individuals were frozen in place during various stages of springing their tails, releasing the furcula and initiating a jump. A specimen at rest would be expected to have all six legs on the substrate and the furcula folded and tucked under the abdomen. The jump consists of the unfolding of the furcula, such that it becomes progressively more extended, the abdomen rises above it, and the legs disconnect from the substrate. Our interest lay in whether or not the collophore

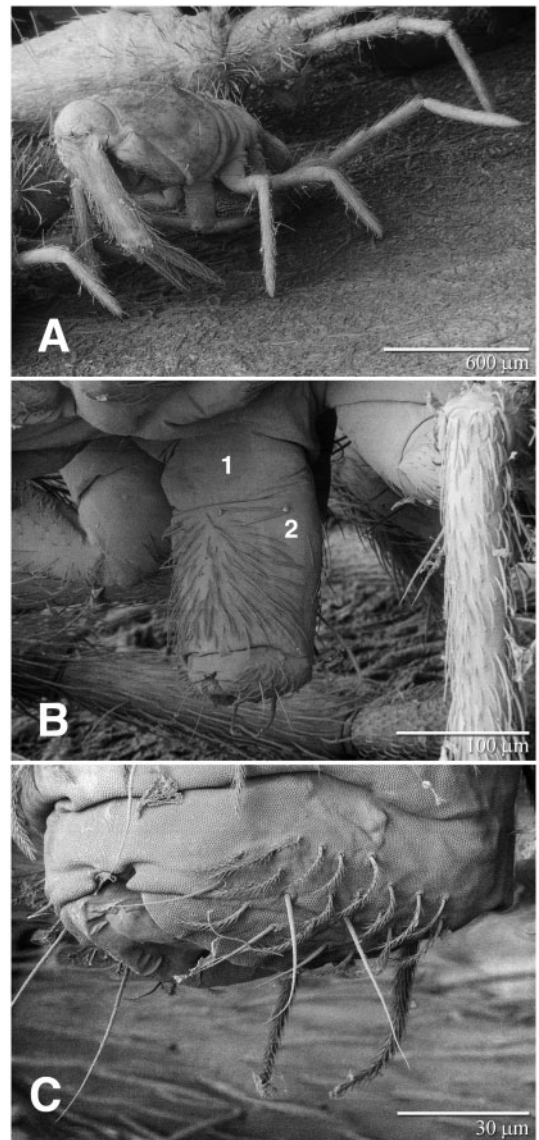


Fig. 2. Springtail initiating jump with collophore retracted. (A) Entire individual. (B) Close-up of collophore with segments 1–2 labeled. (C) Close-up of segment 3; segment 4, the eversible vesicle, is deflated and tucked inside segment 3.

remained in contact with the substrate during this process.

Examining several collembolans caught in the act, some under 3D viewers, it can be seen that the distal surface of the collophore, the eversible vesicle, often remains fixed to the substrate. Figures 3–5 show a progression of stages in the jump. The jump may be initiated with the collophore adhered to the substrate surface (Fig. 3). As the jump initiation progresses and the furcula is partially deployed, the hind end of the abdomen is forced upward, while the collophore remains adhered (Fig. 4). Even after the furcula has

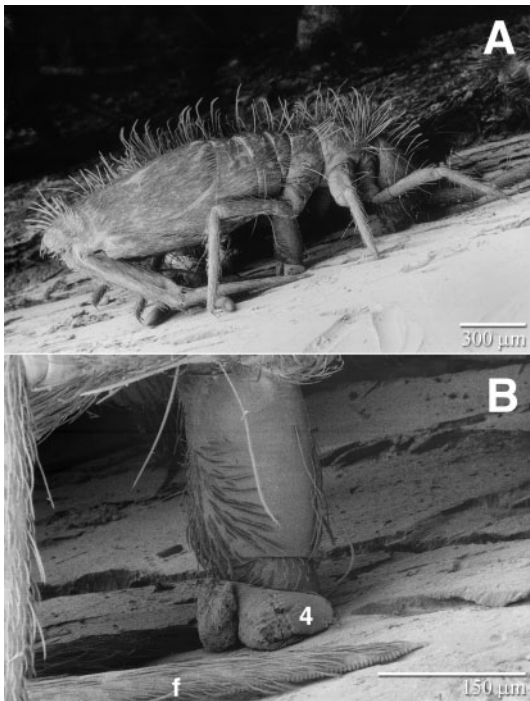


Fig. 3. Springtail before initiating its jump. (A) Habitus. (B) The terminal portion of the furcula (f) is flat on the substrate; the collophore's eversible vesicle (4) is adhered to the substrate.

been extended enough to no longer be in contact with the substrate, the anterior portion of the body may remain in contact with and the collophore adpressed to the substrate (Fig. 5).

Meanwhile, other individuals execute their jump without any apparent involvement of the collophore: they deploy the furcula with the eversible vesicle retracted and the collophore free of the ground (Fig. 2). In at least one instance, the springtail had almost completely executed its jump, but the tip of its furcula remained in contact with the substrate while the collophore remained free of it (Fig. 6).

Discussion

The collophore segments correspond to Hoffmann's (1905) basal plate, tube cylinder, tube collar, and tube vesicle (Fig. 1). Each segment is not independently muscularized, although the fourth does have attachments for retractor muscles; hydrostatic pressure extends the collophore and everts the vesicle (Snodgrass 1993). D'Haese (2004) hypothesized that the entirety of the collophore was homologous to the leg coxa, but the presence of retractor muscles inserted on the eversible vesicle and the serial homology of the collophore, retinaculum (a ventral, abdominal, hook-like appendage that holds the furcula in place), furcula, and legs (Konopova and Akam 2014) suggest that the four collophore sections may each be homologous to specific leg segments. Konopova and Akam's (2014) homeotic leg

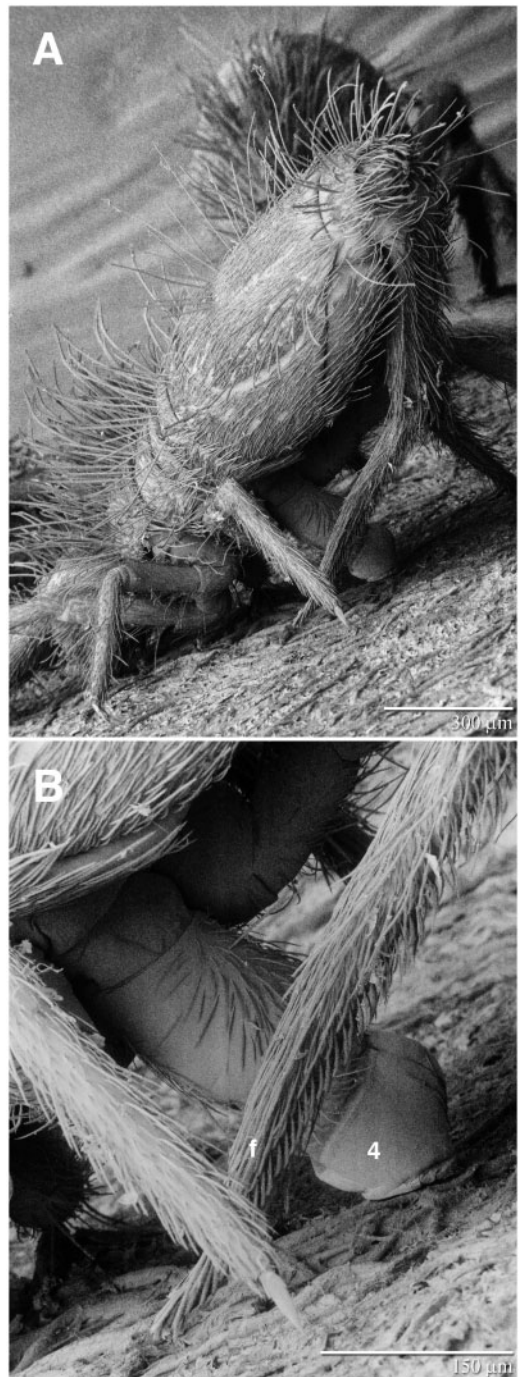


Fig. 4. Springtail initiating its jump. (A) Entire individual. (B) Only the tip of the furcula (f) remains in contact with the substrate; the eversible vesicle (4) remains adhered to the substrate.

in the place of the collophore did grow a tarsal claw, although they did not discuss other possible segment homologies. Derived from ancient legs, the retinaculum and the furcula do still participate in Collembola

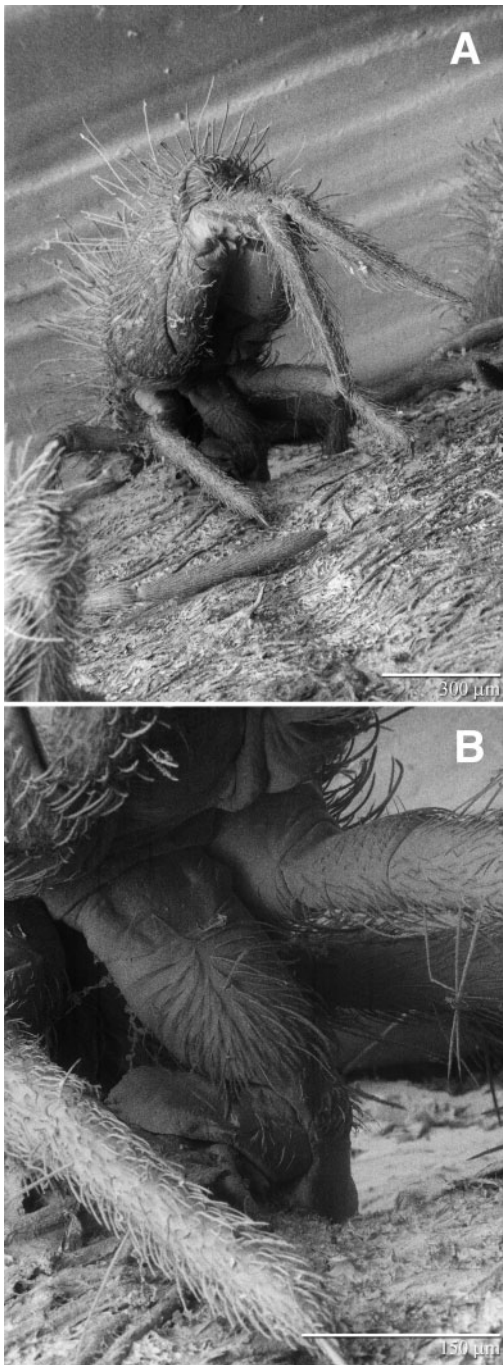


Fig. 5. Springtail with furcula fully deployed. (A) Entire individual; the furcula is no longer in contact with the substrate. (B) The collophore's eversible vesicle is still adhered to the substrate.

locomotion, of a sort; we think the collophore may as well.

Two mechanisms might explain why the collophore remains in contact with the substrate during the jump. In the first, and in our opinion the most likely, a

secreted glue or a physical suction of the vesicles against the substrate, or a combination of the two, keeps the collophore adhered to the substrate. The glue may be produced by cephalic glands and channeled to the collophore via a ventral furrow, the *linea ventralis* (Willem 1900, Hoffmann 1905, Eisenbeis 1974, D'Haese 2004). Also, the collophore's vesicles have a soft and pliable cuticle that permits them to inflate and deflate with hydrostatic pressure (Figs. 1 and 2). The soft cuticle may act to create a seal against the substrate, resulting in a suction-cup effect. If there is indeed adhesive suction, depending on the precise timing, it is conceivable that the force accompanying the eventual release contributes to the force of the jump. However, such a physical or chemical mechanism of adhesion might also hold the collembolan back and dampen the force of the jump. Given the smooth surface of the vesicle (Fig. 4B), nanostructure effects such as van der Waals force is unlikely.

In contrast with the adhesion hypothesis, the collophore may remain in contact with the substrate by being actively extended during the jump. A clear consensus has yet to emerge as to whether the unfolding of the jumping appendage is due simply to muscle contractions in the furcula, the sudden change in hydrostatic pressure inside the collembolan, the release of energy stored in tension in the collembolan's exocuticle, or some combination of the three (Manton 1972; Christian 1978, 1979; Brackenbury and Hunt 1993). Regardless of the principal mechanism for the release of the furcula, it is probable that a dramatic change in hydrostatic pressure does take place inside the collembolan. For example, this sudden change is responsible for the eversion of antennal and other vesicles that aid the landing of the snow flea *Ceratophysella sigillata* (Uzel 1891) at the end of its jump (Zettel et al. 2000). A sudden increase in hydrostatic pressure could cause the collophore to extend and temporarily remain in contact with the substrate even as the rest of the collembolan goes airborne. If this is the case, it is possible that the extension of the collophore contributes to the force of the jump. The collembolan in Fig. 2 appears to contradict this hypothesis, however, as the furcula is released but the collophore is not extended.

As a third possible explanation, we do not consider it likely that the collophore was simply frozen in place before the rest of the collembolan. Had this happened, it might explain why the collophore remains in contact with the substrate even as the rest of the collembolan is in motion for a brief moment longer. However, the entirety of the specimen chamber is flash frozen simultaneously: the copper plates are not cooled prior to the air. Several replicates with modified freezing parameters all produced similar results. Also, some individuals did perform a full jump and some were even frozen in mid-air (Fig. 6): their successful take-off contradicts the idea that they would be frozen in place by the collophore. We also did not observe any torn or damaged collophores that might have resulted had the collophore been frozen while the rest of the collembolan was in motion.



Fig. 6. Stereoscopic images of the springtail in mid-jump. The furcula is fully deployed with only the distal tip still in contact with the substrate; the collopore is free. The reader may cross his or her eyes to visualize in three dimensions the collembolan in mid-air.

We suggest that the collopore, at least in *E. multifasciata*, may be helping the collembolan select the direction of its jump. The standard view is that the length of the furcula largely determines the direction: short furculae provide a forward jump, whereas longer furculae provide a jump where the collembolan flips over backwards (Christian 1979, Zettel et al. 2002). However, several of the individuals in our images each have a long furcula and an elevated posterior abdomen, suggesting the initiation of a forward flip. Christian (1979) reported that *Heteromurus nitidus* (Templeton 1836) was able to select the direction of its jump, preferring to jump in tight angles forward or backward, but rarely straight up. His work did not implicate the collopore. Perhaps the action of the collopore is analogous to that of suddenly applying the front brake on a moving bicycle, causing the rider to tumble over forwards. If this is the case, by coordinating the action of the furcula and the collopore, the springtail can determine if it is to jump forward or backward and perhaps also determine its trajectory. To conclusively test the hypothesis of collopore adhesion and its implication in jump mechanics, we suggest future researchers employ high-speed macro videography to document the Collembola and its collopore in live motion.

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