

Supplementary Materials for

A Triassic-Jurassic window into the evolution of Lepidoptera

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Published 10 January 2018, *Sci. Adv.* **4**, e1701568 (2018)

DOI: 10.1126/sciadv.1701568

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Supplementary Text: Comparative scale morphology

For the taxonomic identification of the fossil scales we studied, in addition to literature information, a series of SEM images (figs. S1 to S5), providing information on scale morphology and structure of extant Lepidoptera, as well as other scale-bearing insects and the non-insect Collembola (springtails). Principal results of this survey are summarized in Table 1. For descriptive terminology of scale characters, we refer to ref. (49).

Collembola: Collembolan scales were studied by scanning the scales from *Tomatocerus vulgaris* of the family Tomoceridae (fig. S1A to D). The scales are relatively thin and are most likely solid. The front side of the scales shows approximately 30 clear longitudinal ridges that extend beyond the apical margin. No cross ridges or herringbone patterns are visible between the ridges. The ridges are not pronounced on the backside of the scale, which is rather smooth (fig. S1C and D). The general shape of the scales is relatively broad and slightly tapers towards the antapical margin. The apical margin is somewhat rounded. Published SEM images of scales from the family Entomobryidae (50) reveal that several species in this family have a scale morphology very similar to that of *T. vulgaris*. They, too, are relatively thin, with clear ridges on top that extend beyond the apical margin, and a relatively smooth backside. Some species, however, have an apical margin that is more irregular in shape. Furthermore, some scales are more elongate when compared to those of *T. vulgaris*. Some species exhibit dentation on the longitudinal ridges.

Archaeognatha: To study the structure of the scales of the Archaeognatha, scales of the archaeognath *Machilis sacra* were imaged (fig. S1E to H). The images reveal that the scales are hollow, since a clear internal structure is visible. This is in agreement with published observations (51). The front side of the scales shows clear longitudinal ridges that are relatively far apart. On some scales these longitudinal ridges extend beyond the apical margin (fig. S1G). Between these ridges there are cross ridges that are also relatively far apart. These cross ridges create rectangular cells that can have a large or small opening in the middle, have two small holes, or can be entirely closed. On the backside of the scale no clear ridges are visible. Instead, the backside is adorned with small round cells (fig. S1F and H). Both the apical margin and the basal margin of the scales are rounded, with a slight tapering towards the basal margin. It should also be noted that the pedicel (point of attachment) of the scale is not located below the basal margin, but slightly above the basal margin of the scale (49).

Zygentoma: Scales of the species *Centolepisma longicaudata* were imaged to investigate the scale structure of the Zygentoma (fig. S2A to F). The scales seem to be rather thin and there is no indication of them being hollow. The general shape tapers toward the basal margin of the scale. The basal margin extends slightly

beyond the pedicel, on both sides of the pedicel. The apical margin is generally rounded but can also be shaped more irregularly (fig. S2A). Longitudinal ridges occur on both the front side and backside of the scales. The ridges on the front side are, however, much closer together than those on the backside (compare fig. S2D and C). On the front side there are cross ridges that can be moderately pronounced (fig. S2B and D) or barely visible. The longitudinal ridges are connected by irregular cross ridges on the backside of the scales (fig. S2F).

Psocoptera: The structure of the scales of the Psocoptera was studied by scanning the scales of two species of psocopterans, one from the family Ampientomidae and one from the family Lepidopsocidae (fig. S2G to H). In both species the front side of the scales is covered with longitudinal ridges that are relatively close together. These ridges extend slightly beyond the apical margin of the scales. No cross-ridges are evident between the longitudinal ridges. The scales have a clear pedicel that is located below the basal margin of the scale.

Coleoptera: The scales of the Coleoptera were studied using SEM images from available literature. Coleopteran scales are rather thick, and the majority lacks longitudinal ridges (52). Those scales that do possess longitudinal ridges, like in species of *Cionus*, show relatively few, thick, ridges (53). Furthermore, cross ridges do not occur on any of these scales.

Kalligrammatidae and Berothidae: Two neuropteran lineages are known to possess scales. A scale of the extinct (Middle Jurassic-Early Cretaceous) neuropteran family Kalligrammatidae was imaged by Labandeira *et al.* (11). This scale is rather elongated and seems to have a rounded apical margin. Longitudinal ridges that do not extend beyond the apical margin are evident, but details regarding the structure of these ridges, or possible cross ridges, are not clearly visible. Despite several sources reporting the occurrence of scales or scale-like setae on the wings and forelegs of some members of the extant neuropteran family Berothidae, only a single SEM image is so far available (11). Like the scale from the Kalligrammatidae, this scale is also elongated and has a rounded apical margin. It is evident that the longitudinal ridges do not extend beyond the apical margin. No details regarding the structure of the longitudinal ridges or the presence cross of ridges are visible. The pedicel of both neuropteran scale types seems to show a slight bulge.

Trichoptera: Traditionally, a lack of scales on the wings is used to distinguish the Trichoptera from the Lepidoptera. Some families of the trichopteran suborder Integripalpia, however, are known to possess scales. It should be noted that this suborder, of which the earliest fossil evidence stems from the Early Cretaceous, is a relatively derived suborder within the Trichoptera (5). Species of the trichopteran genus *Pseudoleptocerus* are known to possess particularly well-developed scales (18, 54). Based on SEM images, the scale structure of *P. chiridensis* was studied in detail by Huxley and Barnard (54). The scales are clearly hollow, and have

perforations on their front side. Furthermore, the scales exhibit longitudinal ridges that may be straight or adorned with ridge-lamellae [*sensu* ref. (55)]. These longitudinal ridges do not seem to extend beyond the apical margin of the scales. No cross ridges are apparent between the longitudinal ridges. The scales taper toward a clear pedicel below the basal margin of the scale, while the apical margin of the scales seems to be somewhat flattened.

Tarachoptera: This extinct order was recently established by Mey *et al.* (12) on the basis of scale-bearing insects included in Late Cretaceous amber. Phylogenetically, Tarachoptera are considered to be basal to both Trichoptera and Lepidoptera. Wing scales are elongated to spindle-shaped, with acute to rounded tips. Relatively dense longitudinal ridges do not extend beyond apical scale margin. The scales are bent and slightly arched, which is in contrast to the flat scales of Lepidoptera. The reported absence of perforations (12) needs confirmation. Unfortunately, SEM images necessary for detecting delicate morphological details, such as perforations, cross ridges, microribs and herringbone patterns, are not available.

Lepidoptera: Since the structure of lepidopteran wing-scales underwent significant evolution during basal family-level divergences (13), scales from different lepidopteran families were studied, including the Micropterigidae, Eriocraniidae, Hepialidae, Nepticulidae, Adelidae, and Tineidae. Scales characteristic of the Micropterigidae, the most basal family within the Lepidoptera, were studied from a member of the genus *Micropterix* (fig. S3A and B). The scales seem to be rather thin and there is no indication that they are hollow. This is in agreement with literature information (10), demonstrating that the scales of the majority of non-coelolepidan lepidopterans are solid. The exception is *Agatiphaga*, the only genus of the family Agathiphagidae, which does possess hollow scales. Given the fact that these scales lack any perforations on the front side, like in the Coelolepida (see below), it is highly probable that the hollow scales of the Agathiphagidae evolved separately and that therefore solid scales constitute the ground plan condition for the Lepidoptera (13, 55, 56). The longitudinal ridges on the scales of *Micropterix* sp. extend beyond the apical margin, and the apical margin itself is rounded (fig. S3A). Between the longitudinal ridges, a herringbone pattern is apparent (fig. S3B). Although not clearly visible in fig. S3B, literature information indicates that there are also cross ridges between the longitudinal ridges (10). Herringbone patterns also occur in the Agathiphagidae and Heterobathmiidae (10).

The other non-coelolepidan of which the scales were imaged was *Eriocrania cicatricella*, a species of the Eriocraniidae. Similar to the scales of *Micropterix* sp., the scales of *E. cicatricella* are also relatively thin and solid (fig. S3C) (10). Furthermore, the apical margin of the scales is also rounded, and longitudinal cross ridges are present that extend beyond the apical margin of the scales. The side of the longitudinal ridges seems to

exhibit ridge-lamellae, whilst the top of the longitudinal ridges exhibits microribs (fig. S3D) [sometimes referred to as flutes, (55)]. Between the longitudinal ridges, cross ridges are clearly visible (fig. S3D). The structure of the scales of the Coelolepida is fundamentally different from that of the non-coelolepidan families. The scales are hollow and have inter-ridge perforations on their front side. Also the presence of serrated apical margins is considered to be characteristic for the Coelolepida (13, 55, 56). The most basal coelolepidan lineage that was studied were the Hepialidae, represented by the species *Triodia sylvina*. The scales of *T. sylvina* exhibit a serrated apical margin (fig. S4A). Furthermore, on the front side of the scales, the top of the longitudinal ridges shows clear microribs. Between the longitudinal ridges, cross ridges are present that are interrupted by fairly large perforations that span the width of roughly three cross ridges. These perforations, which are surrounded by a circular ridge, clearly indicate that the scales are in fact hollow (fig. S4B). The scales of the other coelolepidans that were examined are fairly similar to those of *T. sylvina*. They mainly differ in the size, shape and frequency of the perforations in the front of the scales. The perforations in the scales of *Stigmella malella*, a member of the family Nepticulidae, are moderate in size, oval in shape and occur between every cross ridge (fig. S4C and D). They also lack the circular ridge around the perforations. On the front of the scales of *Adela reaumurella*, a member of the Adelidae family, the perforations are extremely small, lack a surrounding circular ridge and are present between most but not all of the cross ridges (fig. S4E and F). The scales of *Triaxomera parasitella* (Dytrisia; Tineidae) have roughly circular perforations of moderate size between each cross ridge. These perforations also lack a circular ridge (fig. S4G and H). The backside of the scales of most lepidopterans, including those mentioned above, is known to be relatively flat and unsculptured (10).

Culicidae: Scales of the dipteran family Culicidae were studied on the basis of material from the (sub)genera *Janthinosoma*, *Psorophora* (fig. S5A and B), *Culex* (fig. 5C and D), *Culiseta* (fig. S5E and F) and *Aedes* (fig. S6G and H); these taxa provide a reasonable phylogenetic spread (57). The scanned scales show a rounded apical margin and longitudinal ridges on their front side that extended beyond the apical margin. Furthermore, all scales exhibit a clear pedicel below their basal margin. These features are also evident from literature information (58). Observations of the scales of *Aedes* allow for the inference that the backside of the scales of the Culicidae is relatively flat (fig. S5G). On the basis of the cross ridges in between the longitudinal ridges on the front side the scales can be divided into two distinct types, the first of which is most common and occurs in *Psorophora* (fig. S5B), *Culex* (fig. S5E and F) and *Aedes* (fig. S5G and H). This type is characterized by a dense covering of somewhat triangularly shaped cross ridges. A second, less common, type of scale occurs in *Culiseta* (fig. S5B) and *Aedes* (fig. S5G). It exhibits a smaller number of cross ridges that are also less pronounced.

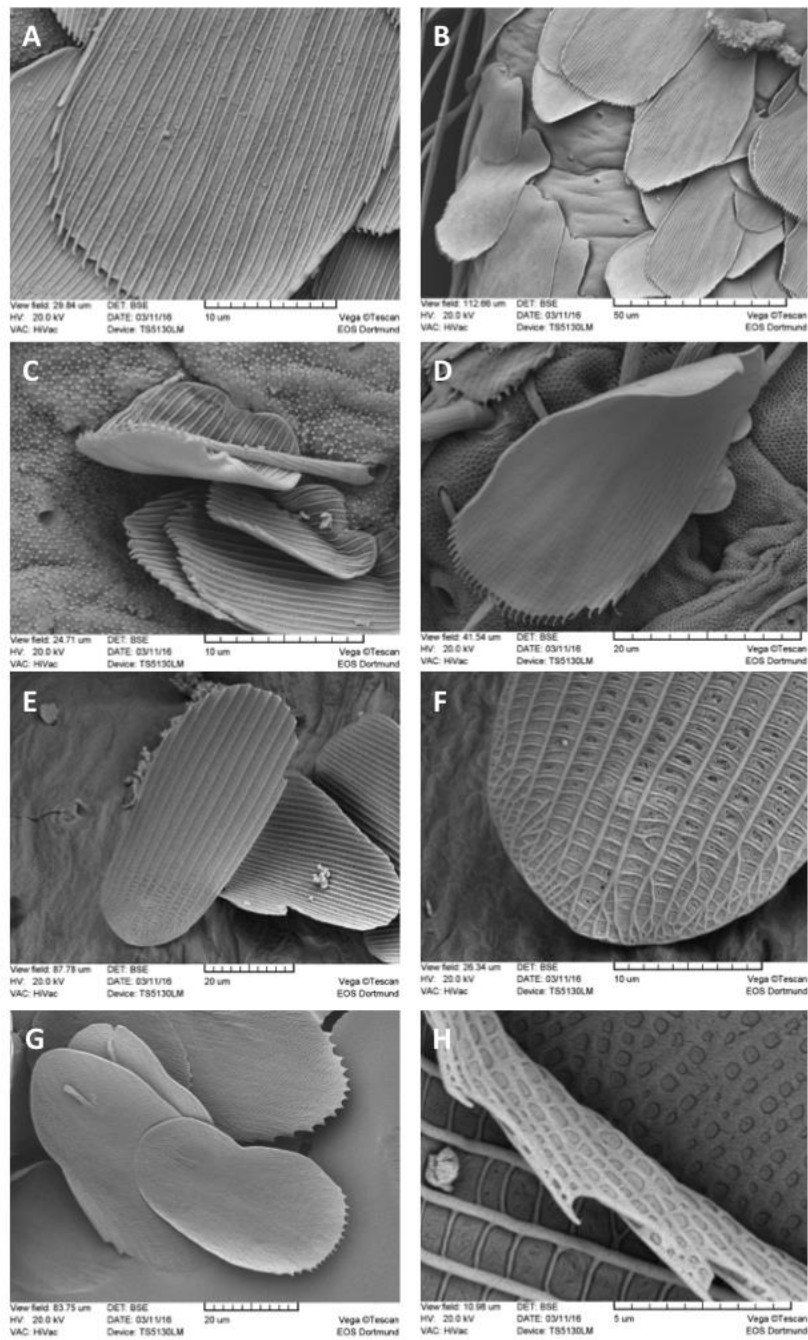


fig. S1. SEM images of scales of Collembola and Archaeognatha. (A to D) Scales of the collembolan *Tomatocerus vulgaris*. (A) Detail of the front side of a single scale. (B) Overview of the front side of multiple scales. (C) Both front side and backside of a single scale. (D) Backside of a single scale. (E to H) Scales of the archaeognath *Machilis sacra*. (E) Front side of several scales. (F) Detail of the front side of a single scale. (G) Backside of several scales, also showing the pedicel of the scales. (H) Image showing both the front side and backside of a single scale.

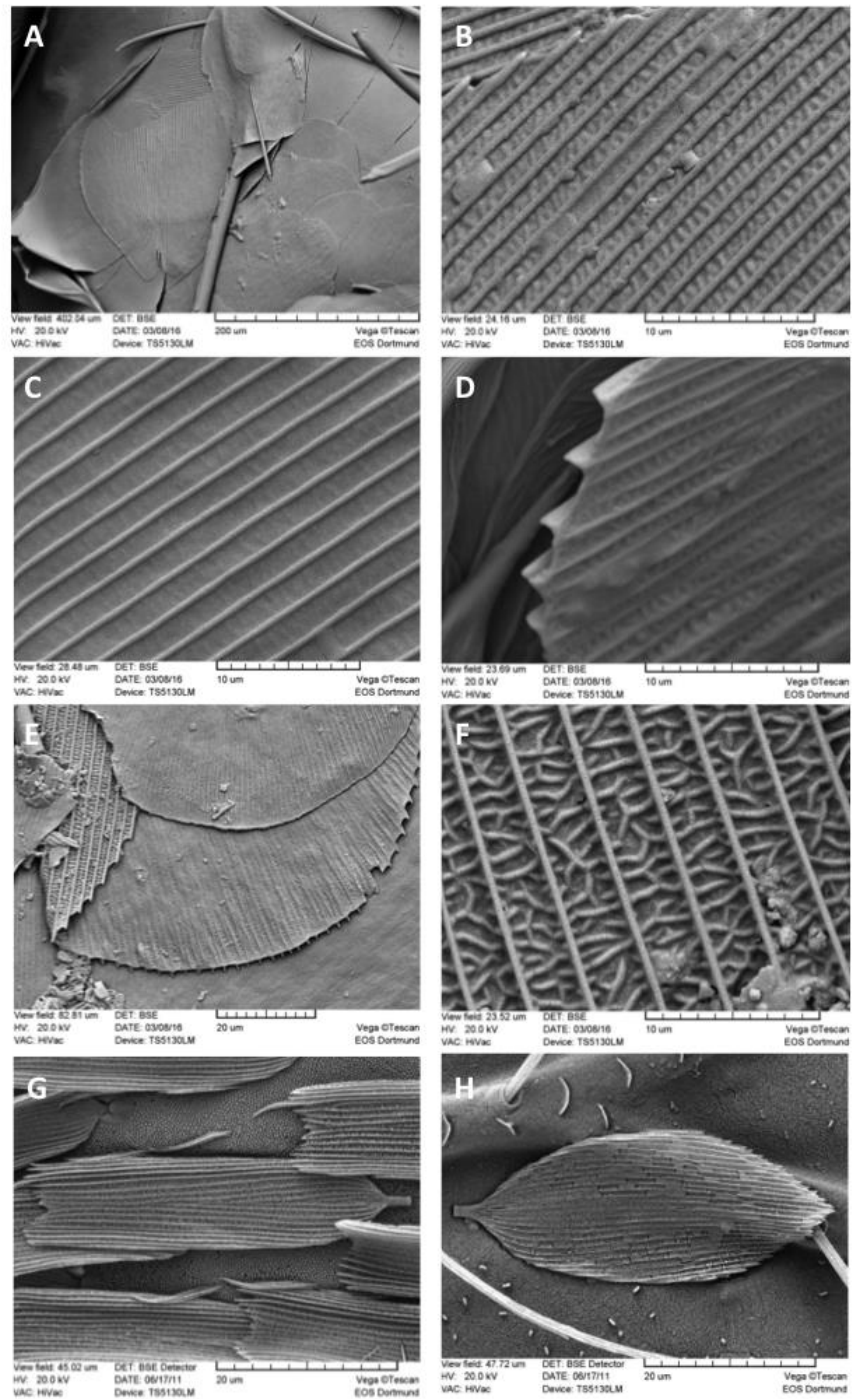


fig. S2. SEM images of scales of Zygentoma and Psocoptera. (A to F) Scales of *Centolepisma longicaudata*. (A) Full scales, front side. (B, C) Details of the front side of the scales. (D, E) Front side and backside of the scales. (F) Detail of backside of a scale. (G to H) Scales of two psocopterans. (G) Scales of a member of the family Ampientomidae. (H) Scale of a member of the family Lepidosocidae.

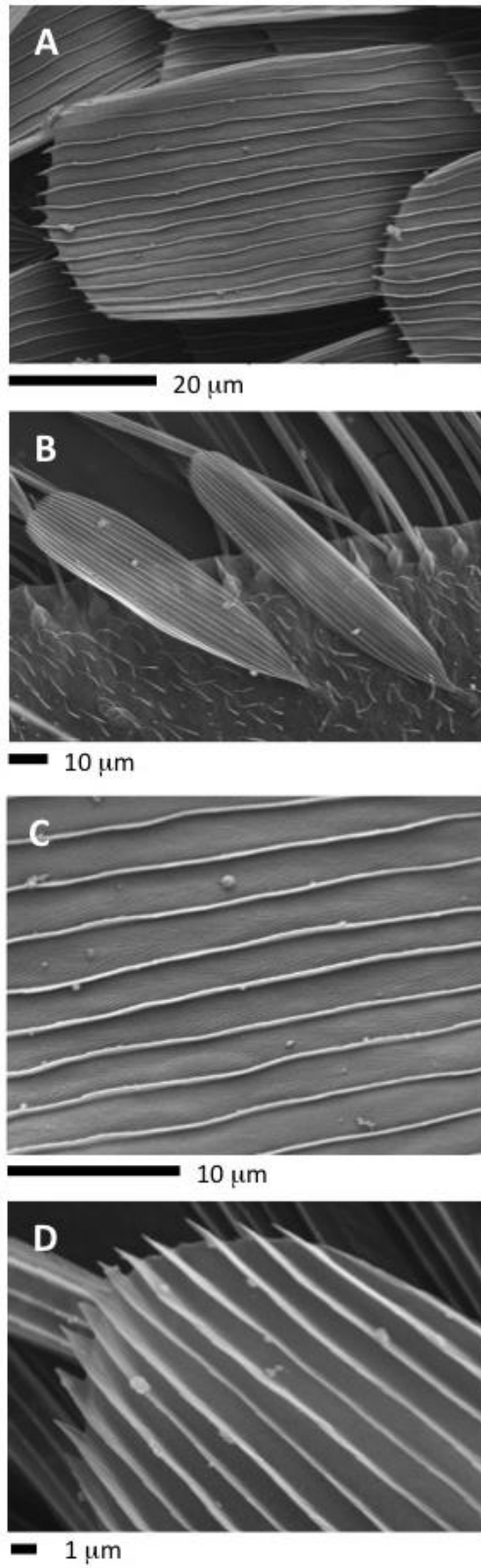


fig. S3. SEM images of scales of non-coelolepidan Lepidoptera. (A and B) Scales *Mycropterix* sp. (Micropterigidae). (C and D) Scales of *Eriocrania cicatricella* (Eriocraniidae).

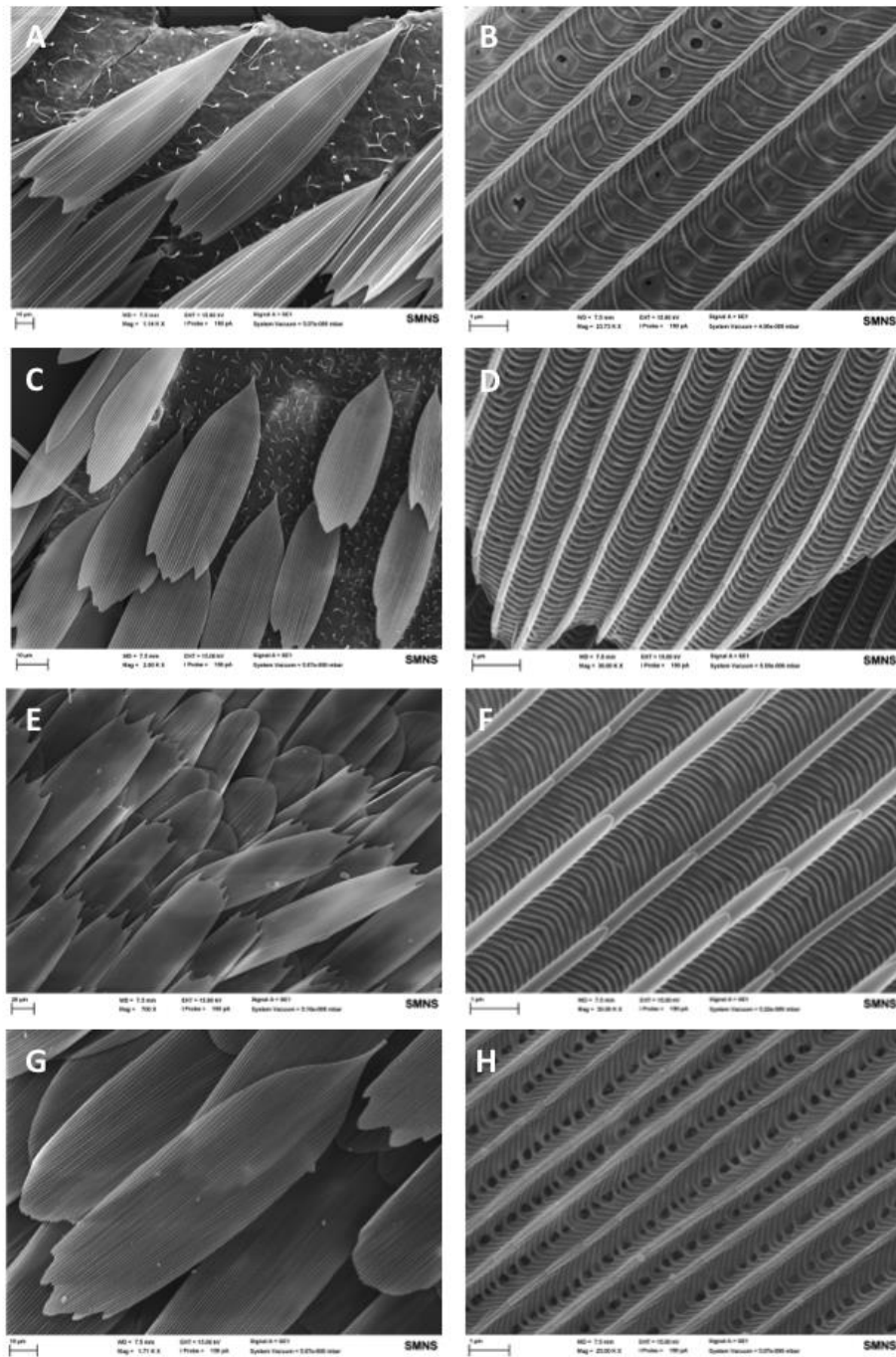


fig. S4. SEM images of scales of coelolepidan Lepidoptera. (A and B) Scales of *Triodia sylvina* (Hepialidae). (C and D) Scales of *Stigmella malella* (Nepticulidae). (E and F) Scales of *Adela reaumurella* (Adelidae). (G and H) Scales of *Triaxomera parasitella* (Dytrisia; Tineidae).

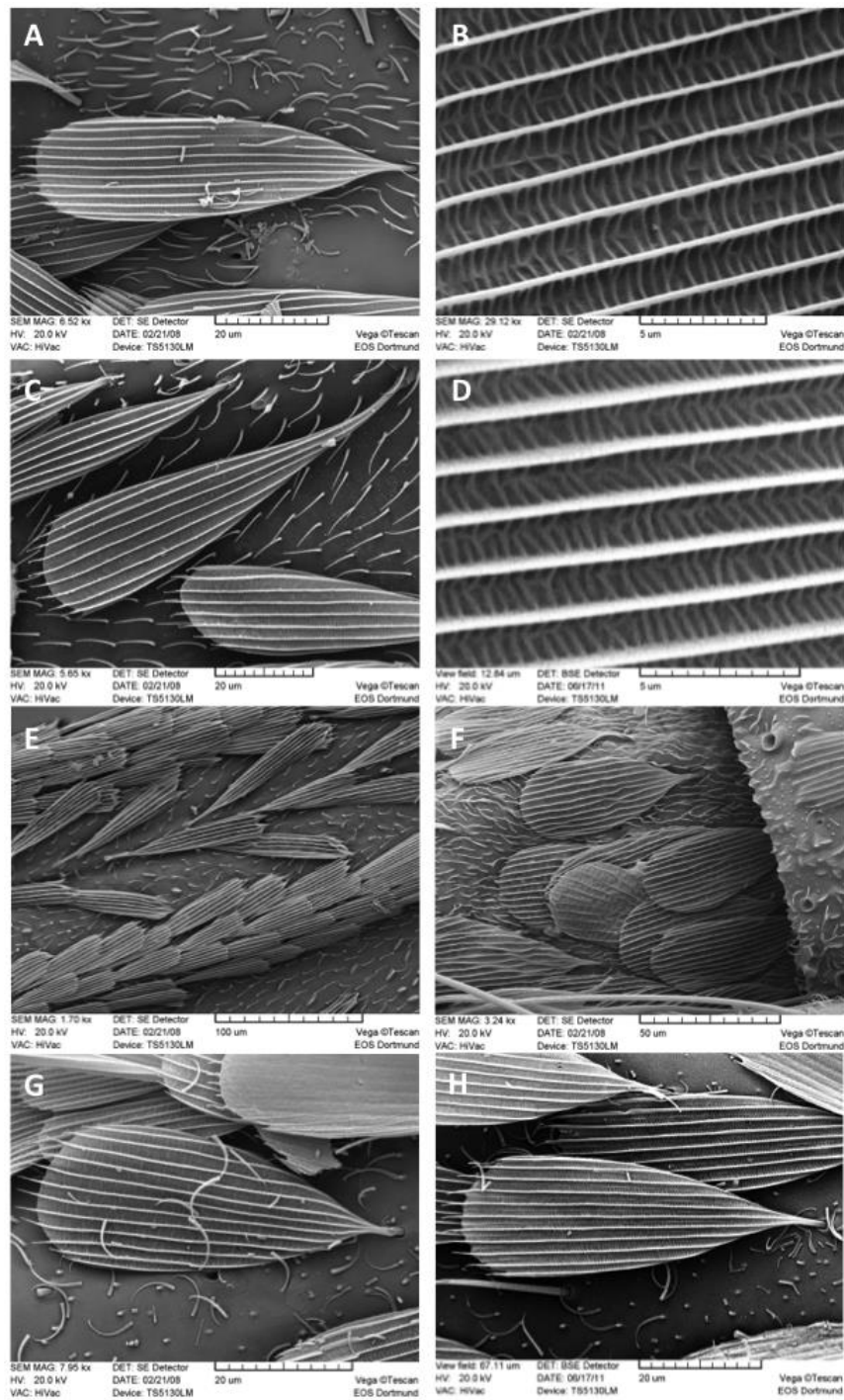


fig. S5. SEM images of scales of Culicidae. (A and B) Scales of *Psorophora* sp. (C and D) Scales of *Culex* sp. (E and F) Scales of *Culiseta* sp. (G and H) Scales of *Aedes* sp.