# MORPHOLOGY, ULTRASTRUCTURE AND FUNCTION OF THE PALMEN BODY AND CONTACT MALLET

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## Abstract

The Palmen body and the similar contact mallet have been incorrectly and incompletely described since their discovery by Palmén in 1877. The Palmen body of a mature mayfly is made of multiple Palmen body units stacked like unique nested boxes, where the latest formed unit (larger than the previous one of the previous instar) covers and overlaps the previous unit. This overlap gives the illusion of rings, where none really exist. Counts of "rings" are then useful in instar determination.

Two-dimensionally, Palmen body formation and structure/ultrastructure are very similar to tracheae, while in three dimensions Palmen cuticle differs greatly. Each unit has multiple uniformly distributed projections that span the interunit space. Each projection is hollow and open on the outer side, as if poking a finger through dough from the outside in. These projections make counting "rings" of whole Palmen bodies difficult or impossible with compound light microscopy because they scatter the light used to view the rings. Palmen bodies appear to lack the inner epicuticle and granular layer that the tracheal cuticle possess.

Palmen bodies and contact mallets are not innervated and probably have no real function, as evident from body and head tracheal comparisons with Odonata.

Key words: Palmen body; contact mallet; cuticle morphology; cuticle ultrastructure; Ephemeroptera cuticle; Ephemeroptera instars; Ephemeroptera; mayflies.

## Introduction

The Palmen body is a cuticular structure that forms the commissure of four head tracheae in mayflies (Ephemeroptera). It is a unique structure known in insects to occur only in the order Ephemeroptera (Landa 1948). The Palmen body apparently is formed at the time of tracheae formation in the second or third instar (Rawlinson 1939, Degrange 1959) and new cuticle is added to the Palmen body during each subsequent instar. The cuticle deposited during different instars appears in the compound light microscope as rings for both whole and sectioned Palmen bodies. The apparent tree-ring like nature of the Palmen body has been infrequently used in determining instars by counting rings (Rawlinson 1939, Degrange 1959, Taylor and Richards 1963, McLean 1970, Benech 1972, Jones 1977, Ruffieux et al. 1996).

The Palmen body was discovered in 1877 by Palmén, and subsequent studies (e.g., Gross 1903, Wodsedalek 1912, Hsu 1933, 1935, Rawlinson 1939, Landa 1948)

have briefly described or figured its basic morphology.  This structure has not been investigated ultrastructurally and only one study (Wodsedalek 1912) seriously considered its function.  These studies did not fully and clearly describe the structure of the Palmen body and major misinterpretations were made concerning basic morphology, formation and function.  For example, the Palmen body is shown in the present study not to be composed of rings at all.  In 1933, Hsu discovered a Palmen body-like structure at the junction of two transverse tracheae in both the eighth and ninth abdominal segments of a *Stenacron interpunctatum* (Say) larva.  Similar structures were subsequently found in other mayflies in the head and abdomen (Rawlinson 1939, Landa 1948, Landa et al. 1980, Landa et al. 1982).  Termed contact mallets by Landa (1948), this term has been adopted in the present study.  Contact mallets have received much less study than Palmen bodies due to their smaller size.

The purpose of this study is to correctly describe for the first time the correct morphology, ultrastructure and formation of the Palmen body and contact mallet.  The apparent function of these cuticular structures was also investigated.

## Methods

*General Dissections and Compound Light Microscopy*.  Due to the very small size of these structures, the very finest watchmaker forceps and the finest minuten insect pins mounted in narrow wooden dowels must be used in all dissections.  In preserved specimens, the Palmen body was best found by locating the connecting tracheae.  First, a portion of the dorsal head cuticle and underlying tissue was carefully removed in the general vicinity of the Palmen body in order to find the connecting tracheae, which served as a guide to the amber colored Palmen body.  The Palmen body tracheae were then cut and used as handles to pick up and manipulate the Palmen body in later processing.  The head should not be pulled from the body since the connecting tracheae and Palmen body may be pulled out of position or the tracheae will be severed.  The Palmen body and its tracheae were much easier to locate in live specimens since these structures were still filled with air and thereby stood out in sharp contrast to the surrounding tissues.  This was greatly enhanced by placing live specimens in 100% glycerin which rapidly cleared in several minutes or more obscuring tissues so that the Palmen body and tracheae may be viewed directly through the dorsal head cuticle.  Subimaginal and imaginal specimens did not adequately clear in glycerin and were dissected.  If the Palmen body was inadvertently separated from its tracheae, then the Palmen body was sucked up into the space between the forceps without being touched.

After dissection, the Palmen body was transferred to a depression slide or regular slide containing a suitable liquid (see below) and any remaining adherent tissues were removed as far as practical.  Whole Palmen body slide mounts for light microscopy were obtained by placing the Palmen body directly on the slide in 80% ethanol, letting the excess alcohol evaporate and then adding a drop of 100%

cellusolve (ethylene glycol mono ethyl ether), and allowing the excess to evaporate before adding 95% ethanol and mounting in euparol. Many other specimens were mounted in euparol without cellusolve clearing. Polyvinyl-lactophenol, cellusolve-balsam, ethanol and 100% glycerin "mountants" also gave satisfactory results; none of these yield permanent mounts including the cellusolve-balsam and polyvinyl-lactophenol, which eventually degrade the Palmen body rings. A dorsal or ventral view of the Palmen body was achieved by laying down flat all Palmen body tracheae. Detailed observations and counting of "rings" were made at 1000 X (oil immersion) using bright-field illumination with or without a green filter. Phase contrast light microscopy was not useful at all due to the complicated multiunit structure of the Palmen body and contact mallet (see results/discussion section).

Sections for light microscopy were prepared by dehydrating specimens in a graded series of ethanol, clearing in xylol, embedding in paraffin, sectioning at 4- to 10- m thickness and slide mounting in xylol-balsam. Staining was with hematoxylin and eosin. Some sections were stained with Mallory's triple stain to determine the layers of cuticle (endocuticle, mesocuticle and exocuticle) (Taylor and Richards 1963, Whitten 1972). All observations and counts were made under 1000X (oil immersion) as above.

*Scanning Electron Microscopy (SEM)*. Whole Palmen bodies for scanning electron microscopy were air dried from ethanol, distilled water or amyl acetate, or critical point dried using amyl acetate/carbon dioxide gas, mounted on aluminum tabs with double sided sticky tape, coated with gold palladium and examined in a Cambridge Stereoscan S4-10 scanning electron microscope at Florida State University. Whole Palmen bodies were manipulated by the attached tracheae. However, if views were desired looking into the Palmen body where the tracheae attach, then the tracheae were pulled off while the Palmen body was wet and the Palmen body was then sucked up into the space between a pair of jeweler's forceps and allowed to air dry on a slide or critical point dried. Minuten pins were then used to prod the Palmen body into a cone up (where a trachea attaches, see results) position. Some dried Palmen bodies were rolled on the double-sided sticky tape by pushing with minuten pins. This served to effectively dissect the Palmen body apart.

Paraffin sections examined in the scanning electron microscope were prepared by dissolving off the coverslip in warm xylol, cutting the glass slide into chips (containing chosen sections) small enough to be mounted on aluminum tabs, rinsing in several changes of fresh xylol, air drying, mounting on metal tabs and coating and examination as above.

*Transmission Electron Microscopy (TEM)*. Palmen bodies for TEM were either dissected out of the head or left in the head. The later proved more successful due to ease of handling. Specimens were then placed in phosphate buffered (pH 7.4) primary fix of 2% paraformaldehyde and 2.5% glutaraldehyde for 1 to 2 hours at

room temperature, postfixed in phosphate buffered 1% osmium tetroxide for 1 to 2 hours at room temperature, dehydrated in a graded series of acetone, and then embedded in Spurr's low viscosity medium.  Thin sections were stained with uranyl acetate and lead citrate and observed with a Phillips 301 transmission electron microscope at Florida State University.

*Procedures for Contact Mallets*.  Procedures used for location, dissection, handling and examination of contact mallets were identical to those used for the Palmen body.

*Function of the Palmen Body: Replicating Wodsedalek's Experiments*.  Wodsedalek's (1912) Palmen body orientation experiments were repeated in the present study on *Maccaffertium exiguum* larvae.  An unsuccessful attempt was made to remove the Palmen body as Wodsedalek reports to have done on nymphs of the similar species *Stenacron interpunctatum* (Say) by using two fine needles (minutens) to pierce and cut a small hole in the head cuticle above the Palmen body, cut the tracheal connections and then lift the Palmen body out.  Much greater success was attained by a procedure that greatly saved time and amount of handling that appeared to be the main causes of mortality.  The nymph was grasped firmly with two pairs of jewelers forceps at the extreme posterior margin of the head, the cuticle was then torn along the coronal suture to a spot just above the Palmen body where a small flap of cuticle was removed, and finally the Palmen body was quickly destroyed by grasping with forceps.  Another attempt involved bending the head downward slightly so that forceps could reach in directly to destroy the Palmen body; however, just bending the head resulted in massive bleeding.

Details of the experiments are described in the results and discussion section for greater clarity.

*Mayfly Specimens Examined*.  The Palmen bodies and/or contact mallets of the following mayfly species were examined in this study: *Hexagenia limbata* (Serville), *Hexagenia bilineata* (Say), *Dolania americana* Edmunds and Traver, *Tortopus puella* (Pictet), *Palingenia* longicauda (Olivier), *Siphlonurus spectabilis* Traver*, Baetisca rogersi* Berner, *Neoephemera youngi* Berner, *Callibaetis pretiosus* Banks, *Baetis* sp., *Caenis macafferti* Provonsha, *Caenis diminuta diminuta* Walker, *Caenis* sp., *Maccaffertium exiguum* (Traver), *Maccaffertium carlsoni* (Lewis) and Heptageniidae sp.  Specimens were either collected by the author in Florida, Alabama, and Louisiana or were obtained from the mayfly collection at the Department of Entomology, Florida A & M University.

*Arthropods Other Than Mayflies Examined for Palmen Body-Like Structures*. Arthropods, other than mayflies, examined for the presence of Palmen body-like structures include the following.  Several specimens of the insect orders Trichoptera, Plecoptera and Megaloptera (*Corydalus cornutus* Linnaeus) were briefly examined throughout the head and body by the glycerin treatment or were clear enough without

glycerin or were dissected. Five fairly mature Thysanura (Lepismatidae) specimens were carefully examined by the glycerin treatment and dissection (glycerin treatment was only partially effective, especially in the head). In the Odonata (all specimens were half-mature to mature larvae), two *Enallagma* sp. (Zygoptera, Coenagrionidae), one *Pachydiplax longipennis* (Burmeister) (Anisoptera, Libellulidae) and two *Celithemis* sp. (Libellulidae) specimens were carefully examined throughout the head and body by the glycerin treatment; the head and body of three preserved *Tramea* sp. nymphs (Libellulidae) were carefully examined and dissected; the heads of mature preserved nymphs of one *Aeschna* sp. (Anisoptera, Aeschnidae), one *Progomphus obscurus* (Rambur) and one *Gomphus* sp. (Anisoptera, Gomphidae) were briefly dissected. Two centipedes (Chilopoda, Scolopendromorpha) and five millipedes (Diplopoda) were examined throughout the head and body by the glycerin treatment and dissection (the glycerin treatment was only partially effective, especially for the millipedes).

### Results and Discussion

*Palmen Body and Contact Mallet Location, Size and Occurrence in Mayflies*. The basic mayfly tracheal system consists of two large lateral longitudinal trunks, one on each side of the body, which run the length of the abdomen and thorax and give rise to branches which supply the gills, caudal filaments, legs, digestive tube, gonads, ventral nerve cord and other areas of the body (Plate 1). Generally in the mesothorax or prothorax each lateral trunk branches (this may occur in the head as shown for *Oligoneuriella rhenana* Irnhoff (Landa 1948) into a dorsal and ventral trunk which supply the dorsal and ventral areas of the anterior thorax and the head.

The Palmen body is located in the head at the junction of four tracheal branches, of the head tracheal trunks, just underneath the coronal suture. In most species it is located near the back of the head but never anterior of the anterior-posterior midpoint of the head (Plate 2).

A small structure, the Palmen body of a mature specimen when measured transversely and longitudinally (measurement directions are in reference to axes of the body and are made from a dorsal or ventral view) ranges in size from 317 by 191 m in a very large mayfly species (*Palingenia longicauda* subimago) to 31 by 27 m and 22 by 19 m respectively for two very small species *Caenis diminuta diminuta* imago and in *Caenis macafferti*.

The Palmen body has been found in all mayflies examined for its presence. This has included over 68 species in 15 families (data gathered from the present study and the following: Palmén 1877, Gross 1903, Wodsedalek 1912, Hsu 1933, Hsu 1935, Rawlinson 1939, Landa 1948, Landa et al. 1980, Landa et al. 1982, Degrange 1959, Taylor and Richards 1963, McLean 1970, Benech 1972, Jones 1977, Ruffieux et al. 1996).

Contact mallets have been found in the eighth and ninth abdominal segments of a *Stenacron interpunctatum* nymph (Hsu 1933), in the eighth abdominal segment of
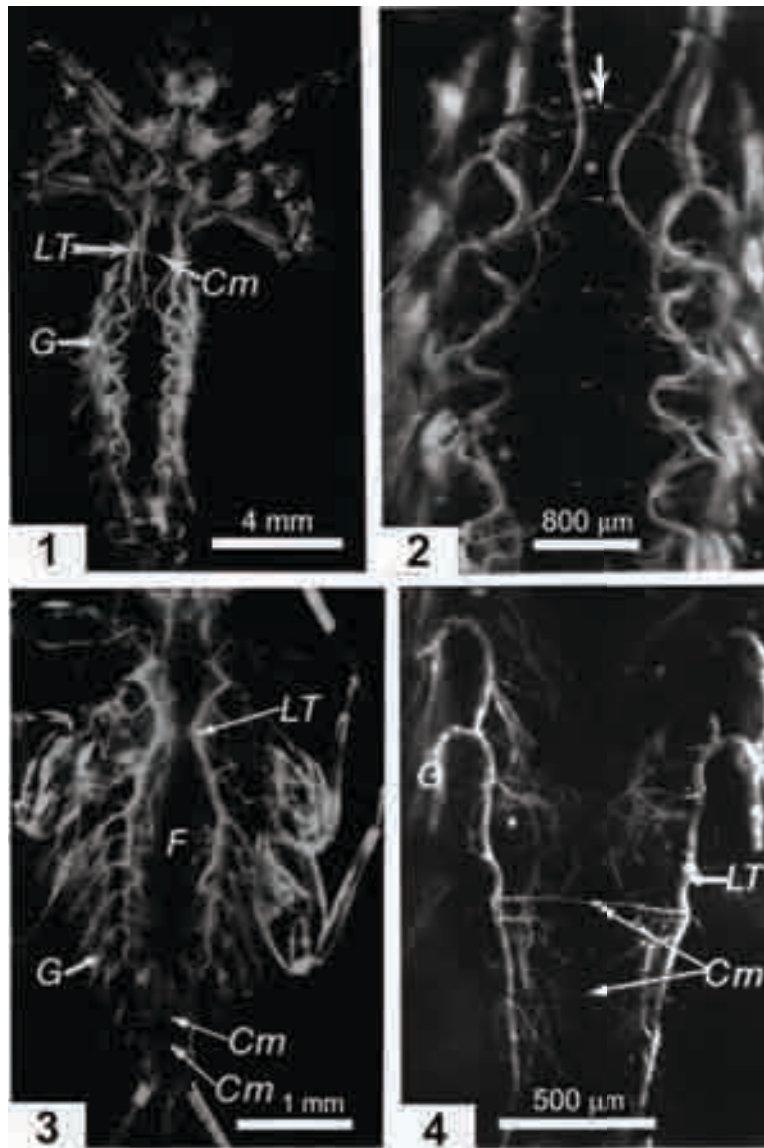
Plate 1: Figure 1—Ventral view of a mature male *Hexagenia* sp. larvae cleared in 100% glycerin. Seven contact mallets are visible just dorsal to the abdominal sternites, where each one forms the anastomosis at the junction of two transverse tracheae in one of the abdominal segments 1–7. Figure 2—Enlargement of Fig. 1. Arrow points to broken contact mallet in segment 2. Figure 3—Ventral view of body of a mature *Maccaffertium exiguum* larvae cleared in 100% glycerin. Two contact mallets visible just dorsal to the sternites of abdominal segments 8 and 9. Figure 4—Enlargement of Fig. 3. (See Appendix A for plate abbreviations.)
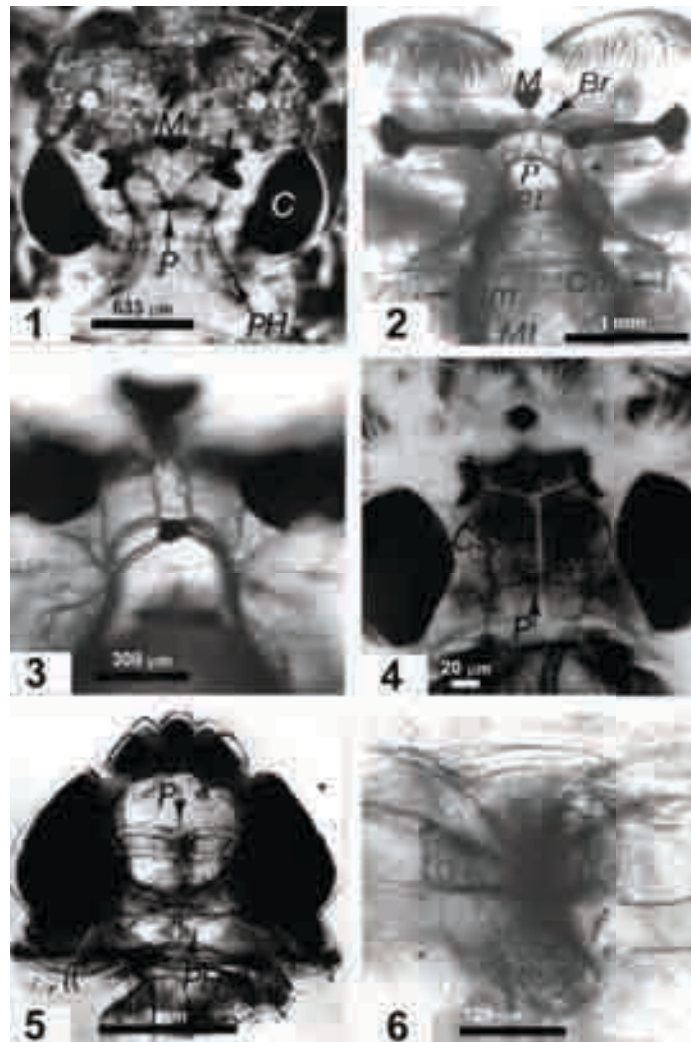
Plate 2: Figure 1—Dorsal view of head of an immature *Maccaffertium exiguum* larvae cleared in 100% glycerin showing location of Palmen body. Figure 2—Dorsal view of head and pro- and mesothorax of an immature female *Dolania americana* larvae cleared in 100% glycerin showing location of Palmen body and contact mallets. Figure 3—Enlargement of Palmen body area of Fig. 2. Figure 4—Dorsal view of head and prothorax of a mature male *Hexagenia* sp. larvae cleared in 100% glycerin showing the location of the Palmen body. Figure 5—Dorsal view of head of a female *Siphlonurus spectabilis* subimago in which much of the dorsal cuticle was removed to reveal the Palmen body and associated tracheae. Figure 6—Enlargement of the Palmen body of Fig. 5. The Palmen body of this species is unique due to the waviness of the rings.

*Ecdyonurus venosus* (Fabricius) (Rawlinson 1939), in other abdominal segments and in the head of a variety of species (Landa 1948).

In the present study, contact mallets were found in the mesothorax of *Dolania americana* (Plate 2: Fig. 2).  In a mature larva of *Hexagenia* sp., contact mallets were found in abdominal segments one through seven just dorsal to the sternites (Plate 1: Figs. 1 and 2).  In a mature larva of *Maccaffertium exiguum*, contact mallets were found in abdominal segments 8–9 just dorsal to the sternites (Plate 1: Figs. 3 and 4).

Landa in his 1948 monograph of comparative mayfly tracheation states that contact mallets are found at all tracheal commissures.  However, in the present study, contact mallets are absent in the ninth abdominal transverse tracheae in a mature male *Baetis* sp. nymph (Plate 3: Fig. 5), and in a mature male *Callibaetis pretiosus* larva (Plate 3: Fig. 6).

Whereas the Palmen body is the anastomosis or commissure of four tracheae, the contact mallet is the anastomosis of two tracheae and is therefore smaller.  The contact mallets seen in Figs. 1–3 of Plate 3 are respectively in size: 53 x 33  m (*Maccaffertium exiguum*), and 59 x 42  m and 77 x 41.5  m (*Hexagenia* sp.), while the respective approximate Palmen body sizes are: 130 x 83  m (*Maccaffertium exiguum*) and 101 x 103  m (or greater, *Hexagenia* sp).  Contact mallets in the abdomen are located in the midline just dorsal to the cuticle of the sternites (Plate 1).

*Palmen Body and Contact Mallet Structure as Viewed in the Compound Light Microscope*.  The Palmen body and contact mallet structure have only been described at the light microscope level.  This is partly why these structures have been erroneously described by all previous investigators.  Examining dorsal/ventral mounts of the Palmen body in Plate 4 from several species appears to indicate a ringed structure, similar to the circuli of a fish scale.  The "rings" appear to have fine lines or striations perpendicular to the running axis of the ring.  In reality the observed rings are really the peripheral area of underlying Palmen body units formed during earlier instars, and the lines are inner projections of the Palmen body units.  This will be become obvious in the next section where Palmen bodies observed under the scanning electron microscope are described.  The projections also create the illusion of a very pebbly appearance on the surface of the Palmen body (Plate 3: Fig. 4) when what one is really viewing are the many projections from the many Palmen body units superimposed on one focal plane.  The attached tracheae never show such a pebbly surface appearance (Plate 3: Fig. 4).

The contact mallet is made identically to the Palmen body except only two tracheae attach to it.  False rings are apparent (Plate 3: Figs. 1–3) and the "pebbly surface" appearance is readily observed (Plate 3: Fig. 3).  Landa (1948) has stated that contact mallets form on the tracheal ends prior to the junction of two transverse tracheae.  This deduction is probably the result of viewing broken contact mallets (see Plate 1: Fig. 2).  Intact contact mallets show a false ring structure over the intact mallet that could not result by fusion of separate halves (Plate 3: Figs. 1–3).
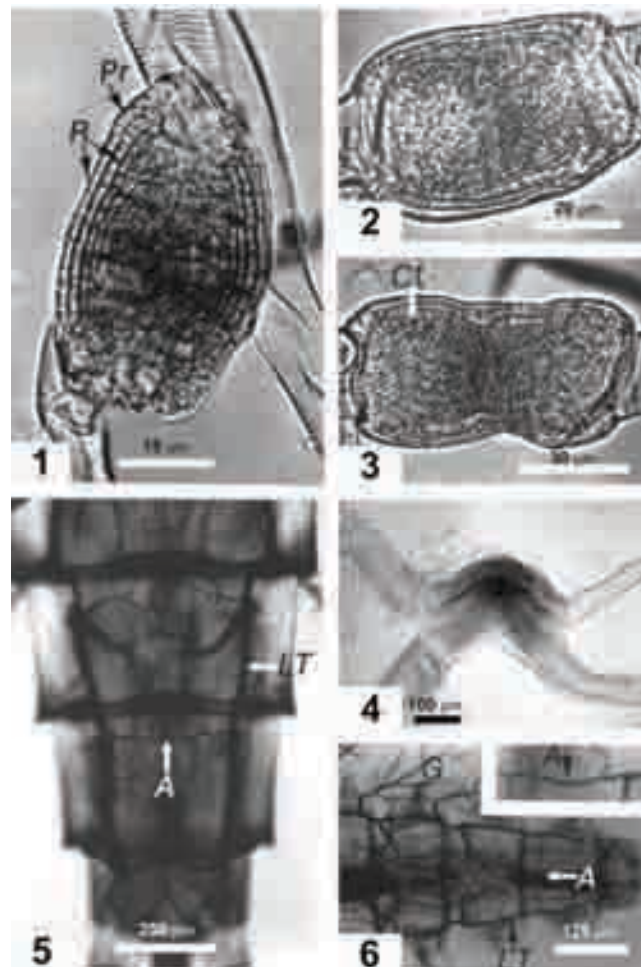
Plate 3: Figure 1—Ventral view of eighth abdominal segment contact mallet from a mature *Maccaffertium exiguum* larvae. Figure 2—Ventral view of fourth abdominal segment contact mallet from a mature male *Hexagenia* sp. larvae. Figure 3—Surface focus on the first abdominal segment contact mallet from larva of Fig. 2. The projections of the underlying Palmen body units superimposed on this focal plane give the illusion of a pebbly surface. Figure 4—Dorsal view of the surface of the Palmen body from a *Palingenia longicauda* female subimago. The surface appears pebbly due to the projections of the underlying Palmen body units. Figure 5—Ventral view of abdominal segments 7–10 of a mature male *Baetis* sp. larvae. No contact mallet is present at the junction of the two transverse tracheae that join together in the anterior ninth segment. Figure 6—Ventral view of abdominal segments 6–10 of a *Callibaetis pretiosus* larvae. No contact mallet is present at the

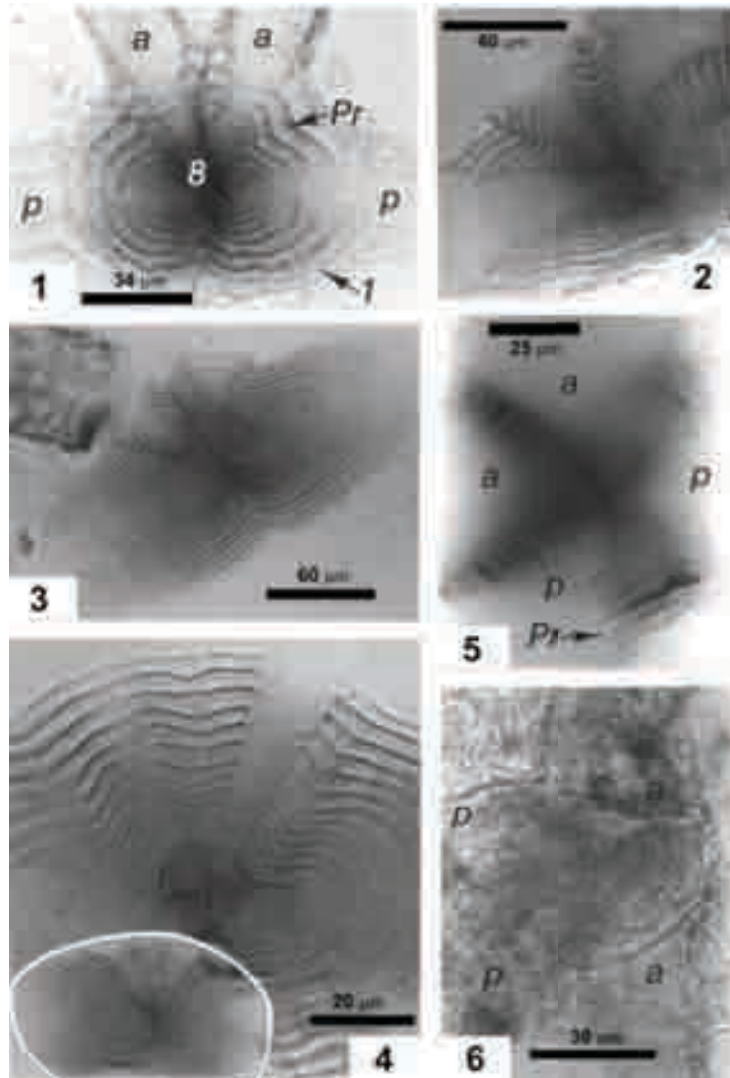anastomosis of the two transverse tracheae which join together in the anterior ninth segment.



Plate 4: Figure 1—Ventral view of a Palmen body from an immature Heptageniidae sp. larvae. There are at least 8 "rings" (Palmen body units) but the center of the Palmen body cannot be clearly seen. Figure 2— Ventral view of a Palmen body from a mature female *Maccaffertium exiguum* larvae. Most of the Palmen body units (apparent rings) can be seen easily; however, the inner units are still difficult to see with assurance. The total number of Palmen body units is 16+. Figure 3—Ventral view of a Palmen body from a Heptageniidae sp. larvae. Most of the Palmen body units are readily visible in the light microscope (although not in this photograph due

to depth of field limitations). The total number of Palmen body units is approximately 17 to 18. Figure 4—Ventral view of a Palmen body from a Heptageniidae sp. larvae (inset entire Palmen body). The "rings" (Palmen body units) of only the two anterior "cones" are quite distinct. The total number of Palmen body units is approximately 15. Figure 5—Ventral view of the Palmen body from a last instar female *Hexagenia limbata* larvae. Total number of Palmen body units is approximately 20 to 21 (not all visible in photograph). Figure 6—Dorsal view of the Palmen body from a mature male *Callibaetis pretiosus* larvae. The overlapping edges of Palmen body units ("rings") are small but distinct and can be counted to the center (but not in this photograph due to depth of field limitations). Total number of Palmen body units is 19 to 20.
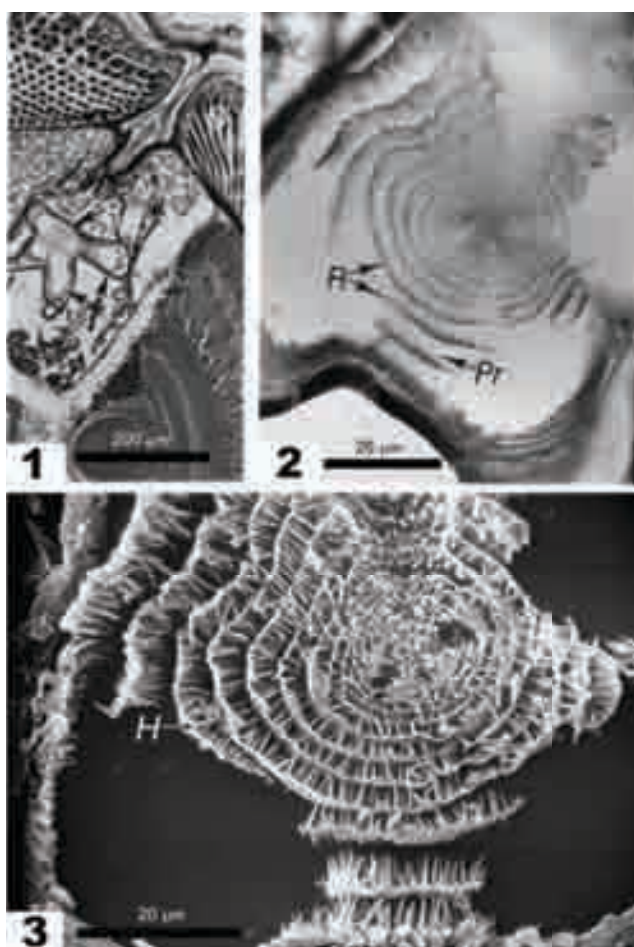
Plate 5: Figures are from 8- m thick paraffin sections from a final instar larvae of *Baetisca rogersi.* Figure 1—Compound light micrograph. Figure 2—Compound light micrograph. Figure 3—Scanning electron micrograph of the section seen in Fig. 2.

*Palmen Body Structure as Viewed in the Scanning Electron Microscope.* Plate 5 shows the Palmen body from a mature larva of *Baetisca rogersi.* Figs. 1 and 2 show a compound light microscope view of a paraffin section showing the four attached tracheae, false rings and false ring projections (Palmen body unit projections). Fig. 3 is an enlargement of the view seen in Fig. 2 but now viewed in the scanning electron microscope. This view indicates about 11 or 12 Palmen body units, which here appear as 11 "rings" and a central mass (Pescador 1974 determined 12 larval instars by morphological examination). The rings here though show that previous descriptions of Palmen body rings were in error in that rings described in previous papers (for the latest account see Ruffieux et al. 1996) are really pointing to the empty space between Palmen body units, and the fine lines or striations on the rings are really Palmen body projections that span the interunit space. These projections are hollow (Fig. 3). Other views of hollow projections in this and other species are seen in the following figures: Plate 6: Fig. 6, Plate 7: Figs. 5 and 6, Plate 8: Fig. 6, Plate 9: Figs. 3 and 4.

Plate 6 shows views of Palmen bodies of *Hexagenia limbata.* The strongly three dimensional nature of the Palmen body is clearly seen in scanning electron micrographs 1–5. The dorsal surface shows a strong chain like circular mesh, which is composed of ridges of cuticle secreted by the former overlying epidermal cells of the same size and shape (Figs. 1, 4, 6). It can clearly be seen where the four tracheae once attached. The Palmen body gives the illusion of being made up of four cone like structures due to the circular lip of the tracheal attachment and the progressively smaller circular lips of inner, earlier formed Palmen body units (Figs. 1–5). Looking through a "cone view" (Figs. 2, 3, 5) shows not rings (which really do not exist) but some of the earlier formed Palmen body units, each one with inward hollow projections. Unfortunately, it is not possible to see all of the Palmen body units from a cone view due to the contorted shape of the units, great depth of the "cones" and the presence of the obscuring projections. Fig. 6 shows the latest formed Palmen body unit from an external view. The holes indicate the beginning of the hollow projections.

The Palmen body units appear to be contorted where adjacent tracheae attach. Here the cuticle arches or deflects towards the center of the unit (Plate 6: Figs. 1 and 4). This can be seen in light microscope preparations (Plate 4: Figs. 1–3, Plate 7: Figs. 1 and 2) and in the paraffin (deparaffinized) thick sections viewed in the scanning electron microscope (Plate 7: Fig. 4).

The Palmen body unit's shape is also affected by the different sizes of the attaching tracheae in at least some species. In *Hexagenia* the posterior tracheae are larger and therefore the Palmen body posterior cones are larger. Like *Hexagenia,* most Palmen bodies are squarish in overall shape. Some Heptageniidae species

mature Palmen bodies, however, are much more flattened dorsoventrally and elongated in the transverse direction (Plate 4: Fig. 3, Plate 8: Figs. 1 and 2).
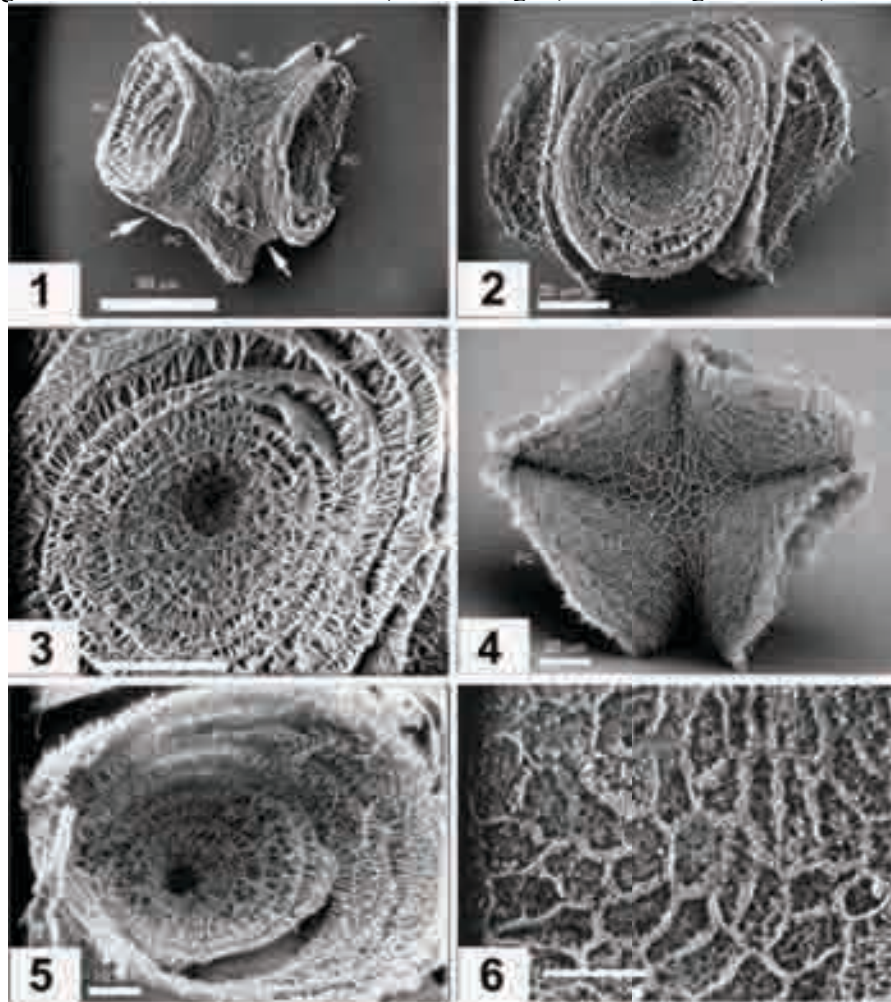


Plate 6: Scanning electron micrographs of the Palmen bodies from two *Hexagenia limbata* female subimagos. Figures 1–3 are from one female. Figures 4–6 are from the other. Arrows in Fig. 1 point to where adjacent "cones" abut (see text).

Many Palmen bodies may be arched dorsally as the Palmen body units are deflected down to meet the upward directed tracheae. This is certainly the case in *Palingenia longicauda* where the large Palmen body can be clearly seen to be arched up dorsally (Plate 3: Fig. 4). This is difficult to see in other Palmen bodies due to their small size. While the edges of most Palmen body units are straight, in the two

Siphlonuridae Palmen bodies examined (Plate 2: Fig. 6, and see Ruffieux et al. 1996) the edges are wavy.
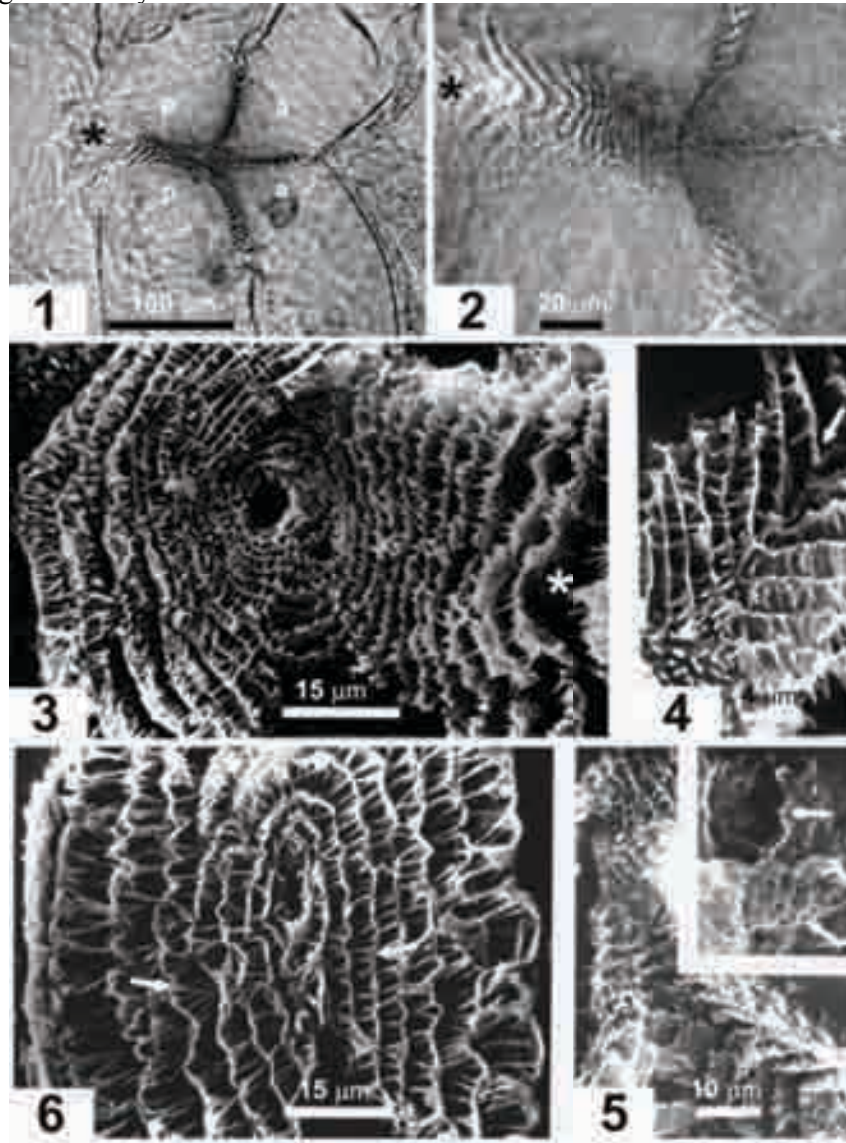


Plate 7: Figures 1 and 2—Light micrographs of the same *Hexagenia* sp. Palmen body at two focuses. * is a region of the Palmen body that is also shown below in Fig. 3. Figures 3, 4, and 5—are 8- m thick paraffin sections from three *Hexagenia limbata* adults (Figs. 3 and 4 are subimagos, Fig. 5 is an imago). Fig. 4 shows the region of the Palmen body where adjacent anterior tracheae once attached. Arrow shows the inward deflection of the cuticle. Fig. 5 shows the hollowness of the projections (arrows).

Figure 6 is a 5- m thick section of a Palmen body from a mature female *Baetisca rogersi* larvae. Arrows point to cuts in projections showing that they are hollow.
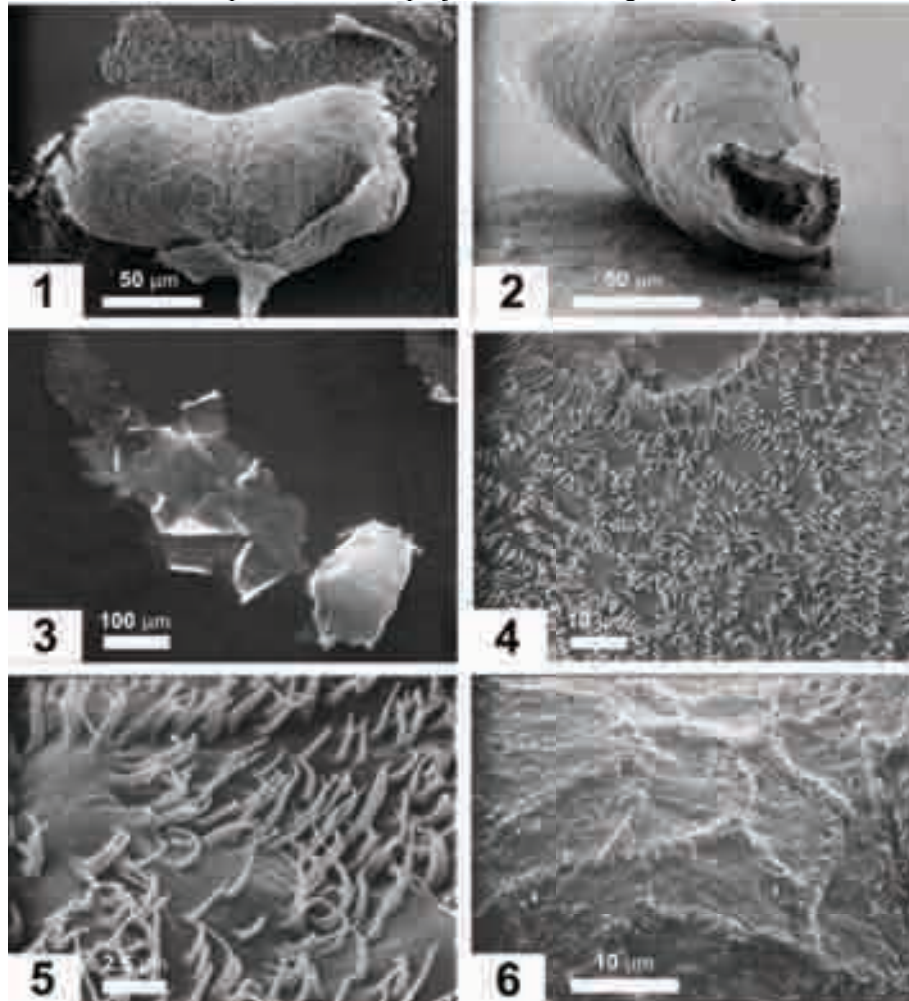


Plate 8: Figures 1–6 are scanning electron micrographs of *Maccaffertium carlsoni imagos*. Palmens in Figs. 1–5 have been rolled on double-sided sticky tape to partially dissect the Palmen body to reveal inner Palmen body units and projections (projections enlarged in Figs. 4–5). Fig. 6 shows the external surface of the Palmen body showing the epidermal cell cuticular ridges and the beginning point of the hollow projections.

In Plate 8 of scanning electron microscope views of Heptageniidae Palmen bodies, you can clearly see the multiunit nature of the Palmen body. In those figures

the Palmen bodies were partially dissected by rolling the body on double-sided sticky tape.  Thus, you can see previous Palmen bodies dissected off and stuck to the tape (Figs. 1–3).  The dissected off Palmen body units also clearly reveal the internal arrangement of the projections.  In these Heptageniidae, the projections occur along the edge of the circular cuticular imprint of the former epidermal cells (Figs. 1–5).  In Figs. 1 and 2, and especially 6, you can see the external cuticular ridge that is the cuticular imprint of the overlying epidermal cells.  Holes on the ridges mark the hollow invagination point of the projections.  In *Hexagenia* the projections are not limited to the periphery of the epidermal cuticular imprint, and appear not to be located on the ridges (Plate 6: Fig. 6).

Scanning electron micrographs of *Dolania americana* Palmen bodies are shown in Plate 9.  Multiple Palmen body units are clearly evident in Figs. 1 and 2. Epidermal cuticular ridges and the external starting point of the hollow projections are seen in Figs. 3 and 4.
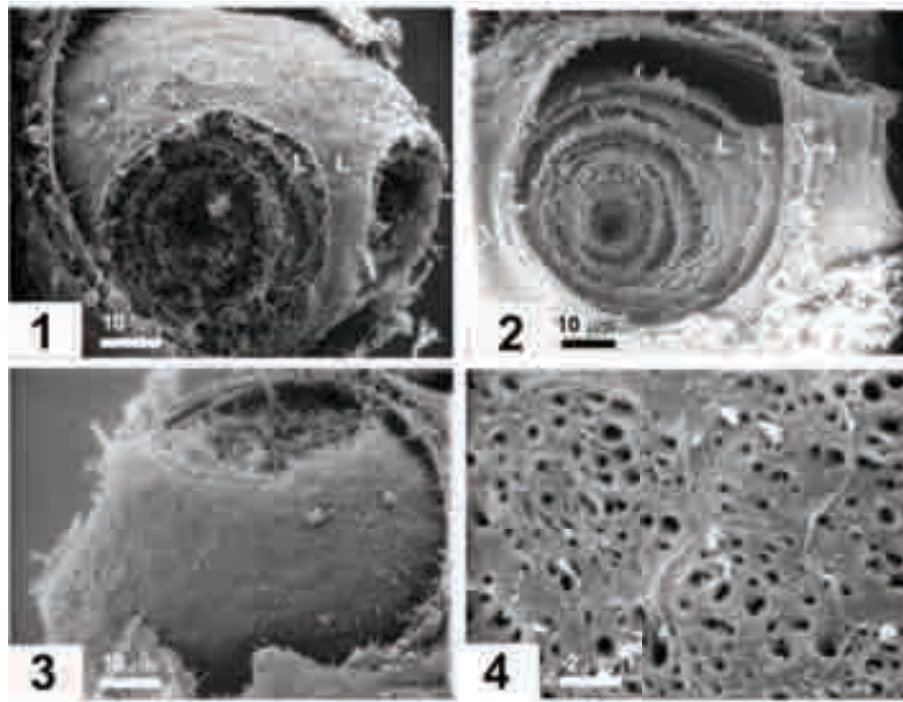


Plate 9: Figures 1–4 *Dolania americana* female subimago Palmen body (same specimen). Figs. 1 and 2 show the multiunit structure of the Palmen, where each subsequent Palmen body unit is a slightly larger facsimile of the one before. Fig. 4 is a highly magnified view of the surface seen in Fig. 3. The dark circular holes are the hollow interior of the projections. The outline of the epidermal cells that secreted the

Palmen body cuticle is indicated by the large circular raised ridges of cuticle that enclose an area with many hollow projections.

*Palmen body ultrastructure.* Light micrographs and TEM micrographs in Plate 10 illustrate for the first time that Palmen body cuticle is modified tracheal cuticle. In Figs. 1–4 you can see the change from tracheael taenidia to Palmen body unit and projection cuticle.
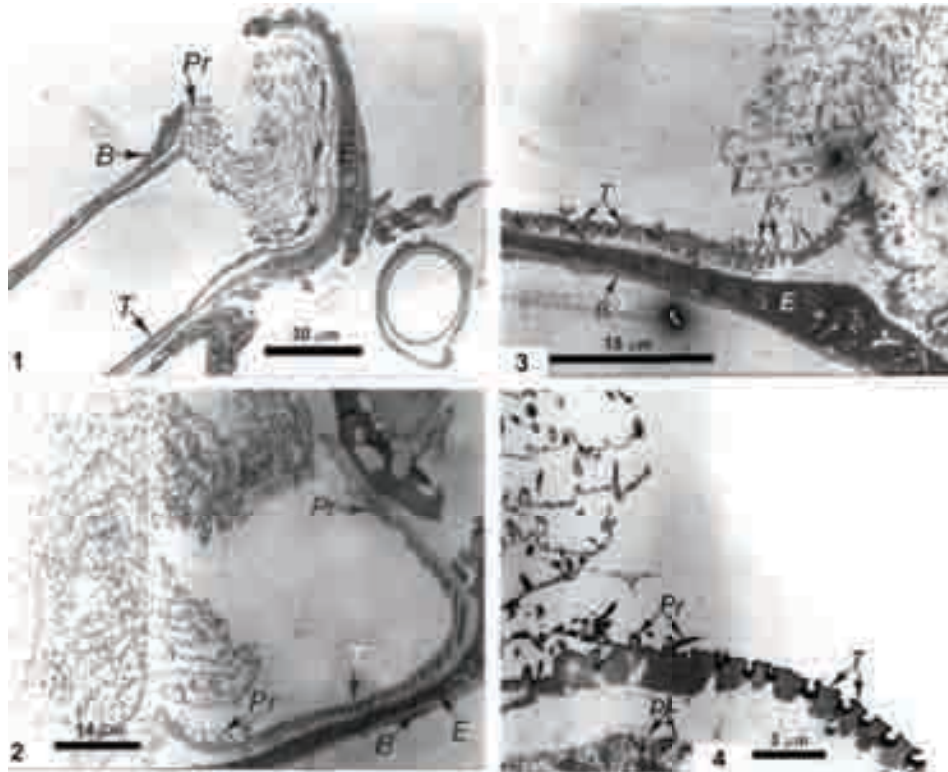


Plate 10: Figures 1–4—Thick (Figs. 1 and 2) and ultrathin (Figs. 3 and 4) epoxy sections through the Palmen body and attached tracheae of a mature female *Dolania americana* larvae, showing the change from tracheal cuticle to Palmen body cuticle. The thick sections are viewed in a compound light microscope, while the ultrathin sections were imaged in TEM.

Contrasting the TEM micrograph plates of tracheae (Plate 11) and Palmen body (Plate 12) shows that ultrastructurally that tracheae and Palmen body cuticle are similar. However, Palmen body epicuticle appears to lack the inner epicuticle and granular layer of tracheal cuticle.

The different three-dimensional molding of these similar ultrastructural components molds taenidial cuticle into Palmen body cuticle. By analogy with the Palmen body, contact mallet ultrastructure would be similar.
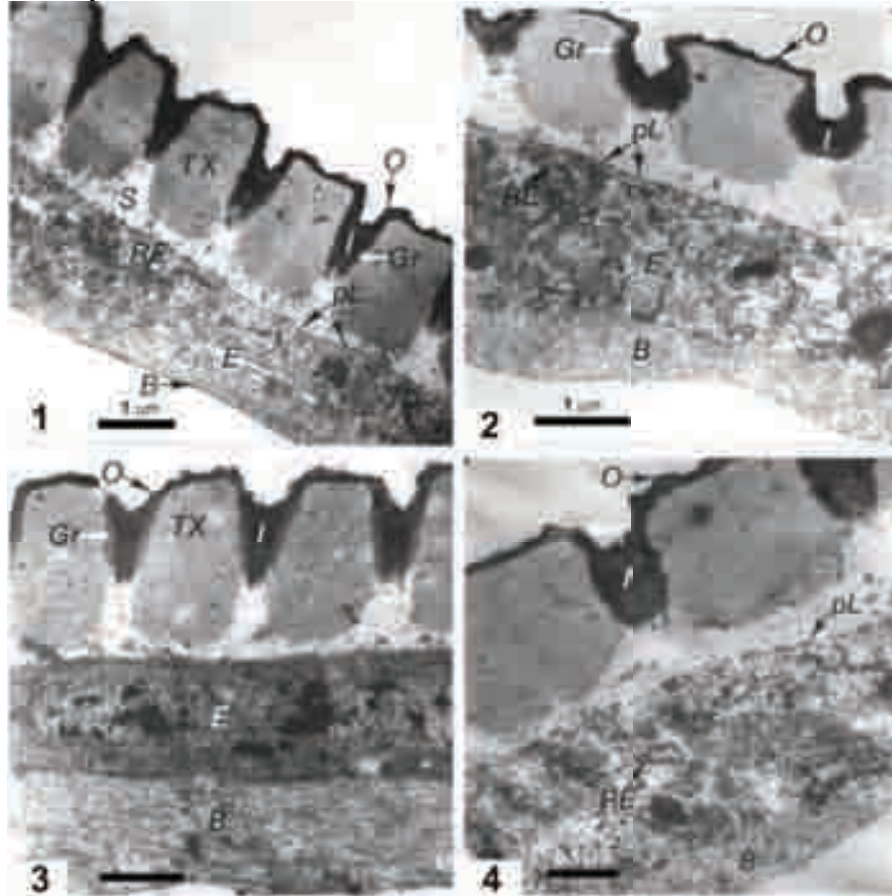


Plate 11: Figures 1–4—TEM micrographs of the Palmen body tracheae from a mature female *Dolania americana* larvae very near emergence to the subimago, showing typical tracheal ultrastructure. Apolysis has begun in this specimen. Compare this Plate with Plate 12 of Palmen body cuticle.

*Palmen Body and Contact Mallet Function*. The fact that all mayflies appear to have Palmen bodies (and certainly many with contact mallets as well) seems to argue for a possible function for Palmen bodies and contact mallets. They may be stronger tracheal junctions than normal tracheal junctions, but broken contact mallets are observed occasionally (Plate 1: Fig. 2), and even in the mayfly most tracheal junctions involve normal tracheal cuticle. A strong argument against a significant

function of Palmen bodies and contact mallets is the lack of these structures in other insects.  Palmén (1877) found no Palmen body like structures in members of the Insect orders Odonata, Plecoptera, Trichoptera, Megaloptera, Diptera, Hymenoptera, Lepidoptera and Coleoptera, and other arthropods.  Plate 13 shows head tracheal cuticle in Odonata that in mayflies would show the presence of a Palmen body. Several specimens of the orders Trichoptera, Plecoptera, Megaloptera and Thysanura were briefly examined in this study and no Palmen like structures were observed.
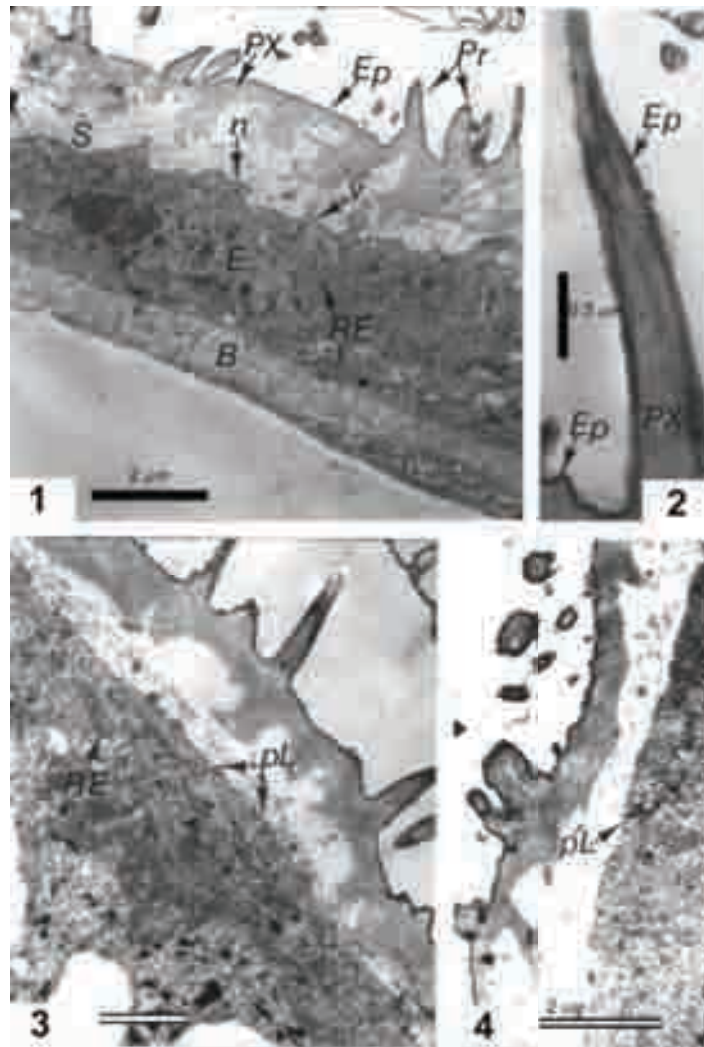


Plate 12: Figures 1–4—TEM micrographs of Palmen body cuticle from the same specimen in Plate 11. Compare with the tracheal cuticle shown in Plate 11. Palmen

body cuticle apparently lacks the inner epicuticle and granular layer of the tracheae. The * indicates a dense staining region that might be molting fluid.
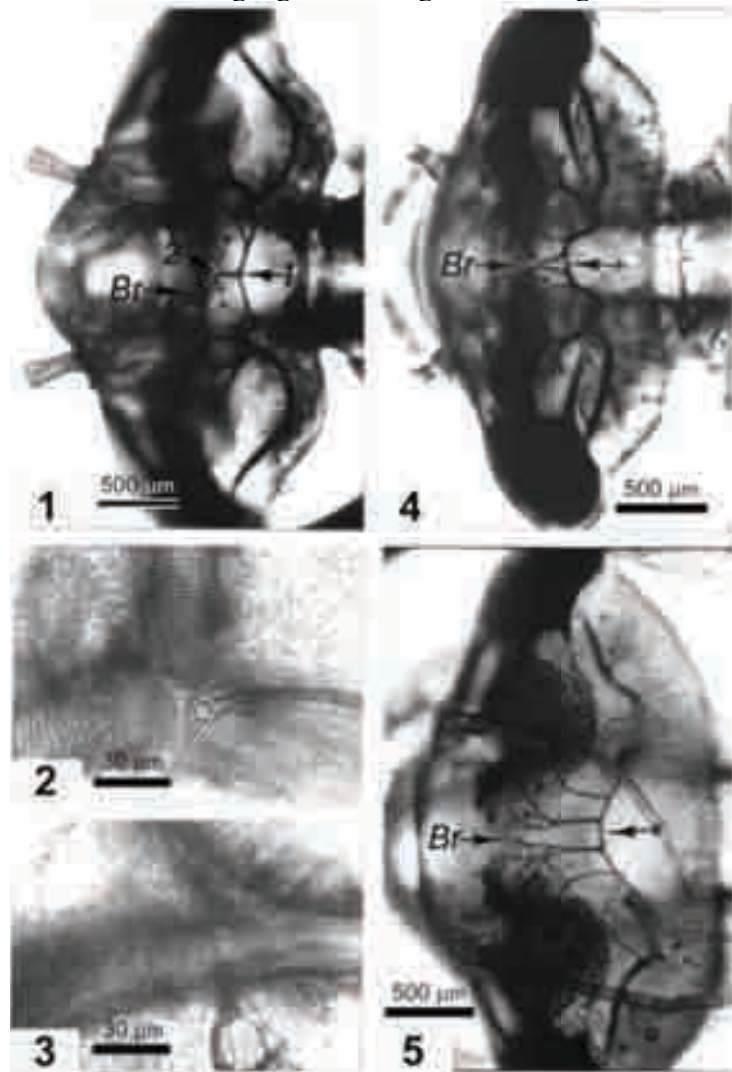


Plate 13: Figure 1—Dorsal view of the head of a mature *Enallagma* (Zygoptera, Coenagrionidae) sp. larvae cleared in 100% glycerin. A non-Palmen body-like tracheal anastomosis (arrow 1) occurs in a location that in mayflies is occupied by the Palmen body. Arrows 1 and 2 refer to areas enlarged in Figs. 2 and 3 respectively. Figure 2—Enlargement of area 1 of Fig. 1. Figure 3—Enlargement of area 2 of Fig. 1. Figure 4—Dorsal view of head of an immature *Pachydiplax longipennis* (Anisoptera, Libellulidae) larvae cleared in 100% glycerin. A non-Palmen body-like anastomosis (starred arrow) occurs in a location that in mayflies is

occupied by the Palmen body. Figure 5—Dorsal view of head of an immature *Celithemis* sp. (Libellulidae) larvae cleared in 100% glycerin. Explanation as for Fig. 4.

Palmén (1877) reports to have found Palmen body-like structures in almost all segments of *Geophilus*, a centipede (Chilopoda, Geophilomorpha). In the present study two live centipedes of the order Scolopendromorpha were examined and no Palmen body-like structures were found, including at the many tracheal commissures that occurred just above the cuticle of the abdominal sternites. Also examined were five live millipedes (Diplopoda) with similar results. However, it was so difficult to dissect the very hard external cuticle of the millipedes that Palmen body-like structures could have easily been destroyed without detection.

Landa (1948) could find no obvious function of Palmen body like structures and stated that perhaps they had some function in molting by providing precise nodes for breaking of tracheal connections so that molting of the tracheae could occur. However, other tracheal junctions in mayflies and in other insects molt quite well without these Palmen body like structures.

Gross (1903) and Brodskiy (1973) proposed, without providing any evidence, an orienting function of the Palmen body during flight.

Wodsedalek (1912) proposed and tested for an orienting-statocyst-like function in larvae of *Stenacron interpunctatum*, which normally inhabit the undersides of rocks. In the first experiment recently dead larvae with and without the Palmen body were dropped in a column of water and larvae with the Palmen body always fell on their dorsal surface while larvae without the Palmen body were equally as likely to fall on either surface. Wodsedalek concluded "..., a self directing process, that is, by the presence of the organ the nymph is swerved into position-a matter of physical equilibrium." Wodsedalek performed two other experiments (experiments 2 and 3) on live larvae, one group (experimental) had the Palmen body removed while the other group (control) had the head capsule similarly dissected but the Palmen left intact. In experiment 2, conducted in a darkened room to avoid phototactic responses, the experimental larvae remained on the tops of the rocks much longer (hours-weeks) than the control larvae after having been dropped in a container of water containing rocks or after the rocks were flipped over. The third experiment was similarly conducted except that rocks were suspended thereby negating certain thigmotactic responses (i.e., body wedging). The experimental group remained evenly scattered over the entire rock surface while most of the control group remained on the lower surface.

I repeated Wodsedalek's experiments using *Maccaffertium exiguum* larvae. This species is in the same family (Heptageniidae) as *Stenacron interpunctatum*, is about the same size, and displays similar larval behavior. In the water-column dead larvae (freshly killed with hot water) dropping experiment, 14 larvae (some several trials with and followed by several trials without the Palmen, while some other larvae were used in trials with the Palmen body and other larvae were used in trials without the Palmen body) were dropped with or without the Palmen body into an aquarium with water to a depth of 122 mm. There was no significant difference between larvae with

or without the Palmen body; out of 59 drops per larval group 39 or 66% in each group fell on their dorsal surface.

In experiment two, three groups of larvae were reared in separate small aquariums with a single rock and larvae were acclimated for 12 hours. The three groups were 36 Palmen body extracted larvae (group E), 33 head dissected but Palmen body intact larvae (group D) and 80 normal larvae (Group N, these were on average smaller larvae than groups E and N since larger larvae were needed for dissection logistics). They were then checked for rock location (top, bottom or sides) eleven times (usually at night) on four days and total survival of groups E and D were noted on the eighth day. Mortality was high for groups E (6 of 36 survived to the eighth day) and D (15 of 33 survived to the eighth day). Larvae were very difficult to see on the rocks and this in itself involved more handling and possible increased mortality, and some nymphs detached from the rocks when the rock was briefly removed from the water for counting of larvae. Despite these problems, more than 80% of the specimens from all groups were found on the bottom of the rocks.

In experiment three, the same three aquariums and the remaining surviving larvae were used, but the same flattish rocks were suspended by rope approximately 8 to 10 cm off the bottom of the aquarium. Six individuals of group E, 15 of group D, and approximately 80 individuals of group N were available for the start and duration of this experiment. Larvae were checked for rock location five to seven times (seven times for group D, and five times each for groups E and N) over three days during both light and dark hours. In all three groups larvae were found on the bottom of the rock greater than 75% of the time.

These observations cast doubt on the validity of Wodsedalek's experiments and strongly indicate that the Palmen body does not have an effect on orientation.

It is extremely doubtful that the Palmen body has any role in orientation for several morphological reasons. First, no nervous connection to these bodies is known (Wodsedalek 1912, Landa 1948, this study). Second, the Palmen body is surrounded in muscle tissue and is not as loosely bound and free to move about as Wodsedalek (1912) stated "It is the writer's opinion that the chitinous organ being so loosely supported by the four tracheal tubes exerts a pressure on the surrounding tissues, whereby the disturbing stimulus reaches the central nervous system." Also the distribution of Palmen body-like structures, i.e., contact mallets, throughout the body appears, as Landa (1948) stated, to argue against an orienting function. Palmen body-like structures may even be a slight detriment to the mayfly since although air can pass through these structures, it must be impeded. However, most probably Palmen bodies and contact mallets are innocuous structures with no vital function.

*The Future of the Palmen Body in Instar Determination of Mayflies.* The Palmen body and rearing are the only accurate instar determination methods in mayflies (Fink 1980, Fink 1982, Fink 1984). The potential of the Palmen body is immense in that it records the number of molts for every individual of any age. Specimens can be analyzed for the number of molts and this information can then be correlated with

developmental stages and with environmental parameters, such as temperature, pollution and relative ecosystem quality. Unfortunately, the very small size of the Palmen body and the multiunit structure hampered further by the presence of projections makes detection/handling and compound light microscopic observation very difficult.

Despite these difficulties, the Palmen body can be used in a very limited way to determine the approximate total number of instars for a species or population. This can be done by simple whole mounts and has worked very well for larger mayfly species in the present study (unpublished data, see the caption for Plate 4).

To unleash the full power of the Palmen body, accurate and fast instar determination of every specimen is necessary. Sectioning of Palmen bodies using TEM epoxy resins is certainly capable of accurate counts (Ruffieux et al. 1996), but it can be argued whether it is a fast method, or even practical for small species/small (young) specimens, or for investigators who lack necessary equipment (e.g., TEM microtomes) and/or skilled labor. In the study cited above, two to three days (including waiting time) were required to process only ten Palmen bodies and this was for a relatively large Palmen for a relatively large species, *Siphlonurus aestivalis* (Eaton).

Confocal laser scanning microscopy is a powerful technique that allows noninvasive optical sectioning of small structures and 3-D reconstruction with appropriate software. Since only focused light is captured, the image is greatly improved over conventional compound light microscopy. While most confocal light microscopy has been done on cells, recently several investigators working on small structures in insects, like genitalia, have used confocal microscopy where images often surpass SEM (see comprehensive paper and references in Klaus et al. 2003). Confocal microscopy could be a powerful tool for instar determination in mayflies if specimen processing and microscope time and analysis can be relatively rapid and fairly foolproof. Large mayfly individuals will require dissecting Palmen bodies out, while very small individuals might not require dissection, and observation of the Palmen body might be possible through the head. Secondly, access to these very expensive confocal light microscopes/computers needs to be routinely available and at an affordable price.

## Acknowledgments

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## Appendix A

### Plate Abbreviations

| | |
|---|---|
| **a** | anterior Palmen body "cone" (where the anterior tracheae attach) |
| **AC** | anterior Palmen body "cone" (where the anterior tracheae attach) |
| **A** | lack of a contact mallet where you would expect one |
| **B** | basement membrane |
| **Br** | brain |
| **C** | compound eye |
| **Cm** | contact mallet |
| **Cs** | coronal suture |
| **Ct** | illusion of a coating due to visualization of the projections from underlying Palmen body units |
| **E** | epidermal cells |
| **Ep** | epicuticle |
| **F** | gut |
| **G** | abdominal gills |
| **Gr** | granular cuticular layer |
| **H** | shows hollow Palmen body projection |
| **I** | inner epicuticle |
| **L** | Palmen body units (appearing like layers) |
| **LT** | lateral tracheal trunk |
| **m** | microvillate apical plasma membrane |
| **M** | median ocellus |
| **Mt** | mesothorax |
| **n** | new outer epicuticle |
| **p** | posterior Palmen body "cone" (where the posterior tracheae attach) |
| **P** | Palmen body |
| **PC** | posterior Palmen body "cone" (where the posterior tracheae attach) |
| **PH** | posterior margin of the head |
| **pL** | plaques of new outer epicuticle |
| **Pr** | Palmen body unit projections |
| **Pt** | prothorax |
| **PX** | Palmen body exocuticle |
| **O** | outer epicuticle |
| **R** | "rings" (the edges of Palmen body units) |
| **Re** | rough endoplasmic reticulum |
| **S** | apolysis space |
| **T** | tracheae (in Fig. 10 refers to taenidia) |
| **TX** | taenidial exocuticle |