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SCIENTIFIC NOTE

PALMEN BODY: A RELIABLE STRUCTURE TO ESTIMATE THE
NUMBER OF INSTARS IN *SIPHONURUS AESTIVALIS* (EATON)
(EPHEMEROPTERA: SIPHLONURIDAE)

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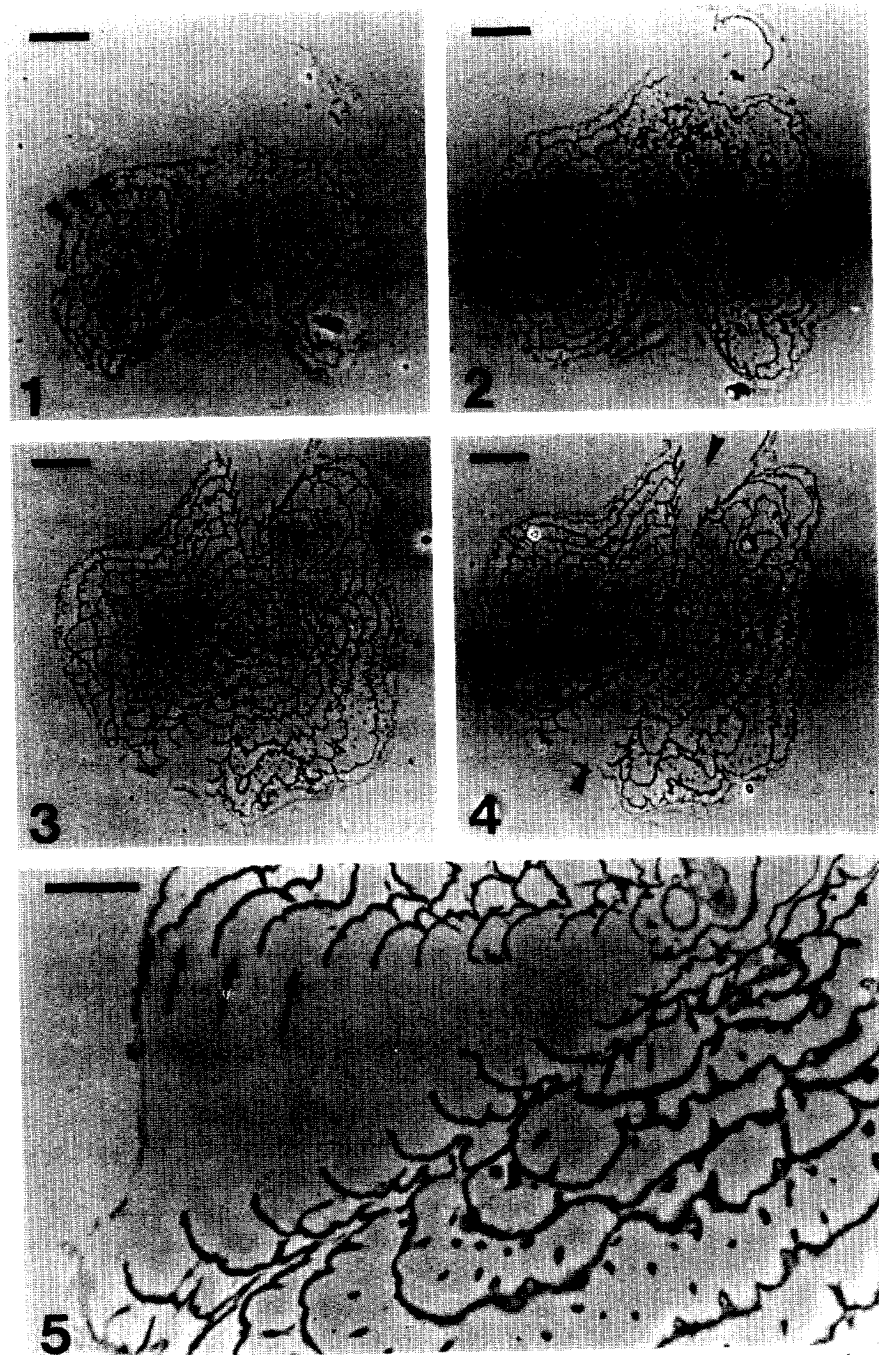
During their aquatic larval life, mayflies (Ephemeroptera) go through from about 10 to 50 ecdyses that represent the largest number of instars among insects (Sehnal, 1985). Eight methods are used to determine the number of larval instars in mayflies, but only 2 direct techniques are reliable: the rearing of individual insects from the egg to the imago and the counting of the successive chitin layers making up the Palmen body (Fink, 1980).

The cephalic Palmen body was discovered by J.A. Palmen in 1877 (in Needham *et al.*, 1935). It is a ringed sclerotized structure in the head of mayflies. Situated between the compound eyes, posterior to the brain, just above the oesophagus, it is made up of the junctions of 4 large tracheae. It consists of several concentric rings of intima, left behind at successive ecdyses, one layer being secreted during each instar. A continuous hypodermal layer surrounds it. Other Palmen bodies are also present in the abdominal segments (Landa *et al.*, 1980, 1982). Even though they also consist of rings of chitin, they are about one-fourth the size of the cephalic ones, because they are formed by the junction of the inner ends of only 2 small transverse tracheae.

This paper describes how to use the cephalic Palmen body to determine the number of larval instars in *Siphonurus aestivalis*.

Fifty nymphs (mature larvae) of *Siphonurus aestivalis* were collected in the river Le Veyron and in the nearby pond Les Monneaux (canton de Vaud, Switzerland), between 2 May and 21 June 1994. They were immediately fixed in 70% ethanol. The head of the nymph was cut longitudinally between the eyes and the cuticle was removed. The Palmen body became visible by pushing forwards the brain. The 4 tracheae were cut and the body was preserved in 70% ethanol. According to the usual steps applied in histology and in microscopy (Nezelof *et al.*, 1972; Locquin and Langeron, 1978), the embedding of the Palmen body was done in Epon 812 (Dodecenyl succinic anhydride:Methyl nadic anhydride:Tridimethylaminoethylphenol, 44.33:12.53:29.63: 1, w/w). It was dehydrated in a graded series of alcohols (95% ethanol for 20 min; 100% ethanol, 3 times for 20 min) and

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Figs. 1-3. Sections of a *S. aestivalis* Palmen body showing the chitin layer (arrows) increase between the successive preparations. Bar = 50 μm .

Fig. 4. Central section of a *S. aestivalis* Palmen body. Two tracheae are longitudinally cut by the section (arrows). Bar = 50 μm .

Fig. 5. Trachea of a central section of a *S. aestivalis* Palmen body showing the 12 successive chitin layers (arrows). Bar = 20 μm .

100% 1,2-propylene oxide (twice, 30 min). It was then impregnated for 60 min in a solution of Epon 812:propylene oxide (1:1, v/v) and finally soaked in Epon 812 (12 h).

For each species, it is necessary to test the thickness of the first sections: some of the cuticle layers can be missed if the preparations are too thick, but examination of too many very thin sections is tedious and impractical. An adequate thickness allows about 5 sections to cut before the next ring appears; if a section is bad or damaged, several sections are still available.

The Palmen body of *Siphonurus aestivalis* is more or less spherical, measuring 200–300 μm in diameter and a section thickness of 0.75 μm was used to analyse it. The sections were mounted on a microscope slide in Canada Balsam and observed with a light microscope. The best picture was obtained in phase-contrast.

Interpretation of the first sections may be problematic (Fig. 1), but a layer increase was always distinct between the successive preparations (Figs. 1–4). The closer the Palmen body center was, the more distinct the layers became. With a magnification of $600\times$, each layer was distinguished, even though the interpretation was easier with open rings (Fig. 5). Only 8% of the Palmen bodies were illegible, the layers being not distinct.

The number of layers making up the *S. aestivalis* Palmen body varied between 10 and 17.

The environmental conditions, especially the water temperature (Vannote and Sweeney, 1980; Rosillon, 1988; Giberson and Rosenberg, 1992) and the food (Cianciara, 1979) affect the growth rate and the number of larval instars in mayflies. Information on the number of instars could clarify some important phenomena of the mayfly life history, such as the size differences between individuals of different cohorts or generations.

Several authors used the Palmen body to estimate the number of larval instars in mayflies (Rawlinson, 1939; Taylor and Richards, 1963; McLean, 1970; Benech, 1972). Degrange (1959) was the first to prove that the number of chitin layers making up the cephalic Palmen body was correlated with the number of instars. He reared some *Cloeon simile* from the egg to the imago, and compared the number of ecdyses with the number of Palmen body layers; he found the same values. Fink (1980) suggested that thin sectioning was the preferred technique to count the Palmen body rings. He also mentioned that the Palmen body method presented technical difficulties due to the size of the body, such as the power of resolution of the light microscope that could be insufficient to distinguish the inner rings of a small Palmen body. If the Palmen body of *Cloeon simile* is considered as one of the smallest with a diameter of 60 μm (Degrange, 1959) that is to say, 3–5 \times smaller than that of *S. aestivalis*, it means that 0.2 μm -thick section would be necessary so as not to miss some layers. These thin sections can be examined in light microscopy, and if the inner rings are close to the limit of resolution of the microscope, it is possible to contrast the preparations with a stain containing heavy metals to observe them in electronic microscopy. So, this method is relatively rapid, easily applicable in the laboratory, reproducible and above all efficient. Even though 2–3 days of manipulations are required to analyse 10 Palmen bodies, it is much faster than the rearing from the egg to the imago; furthermore, only one sample of mature larvae is necessary. Moreover, this method is successful in more than 90% of the cases.

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