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Mesocyclops thermocyclopoides (Copepoda: Cyclopoida):A Scanning Electron Microscopy Study

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ABSTRACT. The copepod *Mesocyclops thermocyclopoides*, a species used in the biological control of the mosquito *Aedes aegypti*, is described using scanning electron microscopy; pictures of the 4th and 5th pair of legs, the maxillulae, maxilipeds and maxillas were taken.
Keywords: *Mesocyclops thermocyclopoides*, ultrastructure, scanning electron microscopy, mosquito control, biological control.

RESUMEN. Se describe el copépodo *Mesocyclops thermocyclopoides*, una especie usada en el control biológico del mosquito *Aedes aegypti*, empleando microscopia electrónica de rastreo; se tomaron fotografías de los pares de patas 4 y 5, las maxilules, maxilipedos y maxilas.
Palabras clave: *Mesocyclops thermocyclopoides*, ultrastructure, scanning electron microscopy, mosquito control, biological control.

INTRODUCTION

Copepods are widespread in ponds, lakes, streams, and small reservoirs in tropical and subtropical regions. The first description of copepods in Costa Rica was reported by Picado (1913) in the water puddles caught in bromeliads, that he called "aerial swamps".[11] Seventy four years later, Collado *et al*. (1984) reported a systematic description of copepods from fresh-water bodies in Costa Rica and indicated that the genus *Mesocyclops* (Harada) was endemic.[1] Thirteen years later, this species was selected as a means of biological control for the Dengue-fever-moquito *Aedes aegypti* in Costa Rica.[5]

Although copepods were identified as predators for other fresh-water inhabitants, such as mosquito larvae, since Fryer, 1957,[2] their importance in biological control was recognized until 1976. The usefulness of copepods in the control of mosquitoes was discovered thanks to an observation made by Riviere and Thirel, when they found copepods in the water of some ovitraps that never obtained mosquito larvae; these ovitraps had been filled with water from a river. These authors evaluate and confirmed their potential for biologic control against mosquitoes in a field survey during 18 months.[13] Today, at least nine species of copepods are used in this way. There are reports of their effectiveness under laboratory conditions and in large scale field experiments; for example, Marten *et al*. ,1997 have been pioneers in controlling *Aedes* in piles of tires in New Orleans.[7] In another large scale intervention in a rural village of Vietnam, *Aedes aegypti* was eradicated after an integral plan, in which the biologic control was achieved by the use of copepods. [9]

The species of copepods most often used in biological control are *Macrocyclops albidus* and *Mesocyclops longisetus.* Also, in Honduras was used *M. thermocyclopoides*.[8] We evaluated the later species in laboratory experiments and in the field, concluding that each copepod could kill an average of 7.3 first-instars larvae of *Aedes* per day.[5]

In the past, copepods were usually described and taxonomically classified using methods that require considerable skills to dissect the animal.[6] The animal had to be fixed in alcohol, transferred to glycerin and then dissected under a stereoscope. Using fine needles, the abdomen had to be separated from the thorax. During this manipulation, the specimens might become infringed in essential body parts of the animal like the 5th pair of legs (P5) or the spines of the 4th pair of legs (P4). Dissections at the head of the copepod can also result in loss or damage of maxilla and other mouth parts. Also, the observations made by light-microscopy sometimes lacked in precision. For this reason, Holynski and Fiers used scanning electron microscopy (SEM) for observations on the mouth parts of *Mesocyclops thermocyclopoides*.[3]

Due to the importance of this copepod in mosquito control campaigns, it is necessary to achieve further knowledge about this species, including its ultrastructural characteristics, which is the aim of this paper.

MATERIAL AND METHODS

Copepods were recollected in San José, Costa Rica and cultured under laboratory conditions according the methodology of Marten *et al*.[8] After determining the species using the descriptions provided by Collado *et al*.[1], Petrovski[10] and Reid,[12] 20 adults of *M. thermocyclopoides* were fixed with Karnowsky fixative solution (2,5% glutaraldehyde and 4% paraformaldehyde) for 2 h, postfixed with 1% osmium tetraoxide and dehydrated through an ascending ethanol gradient (30 to 100%), critical point dried, mounted on aluminum stabs, gold ion sputtered and analyzed under scanning electron microscope (Hitachi S-570).

RESULTS

At scanning electron microscope the observation of *M. thermocyclopoids* (Fig.1), including the P5 is easy and in most cases it was not necessary to dissect the copepod to observe it. This structure identifies the genera *Mesocyclops*: in this case it is biarticulate, the spine at the distal end is long and inserts at the inner margin (Fig. 2A). The observation of P4 was sometimes difficult, although details like the basal plate with their tubercles and the setae found at the outer margin of the basal plate could be observed better, compared to light microscopy (Fig. 2B).

On the abdomen, the lack of setae on the inner section of the furca, marks a characteristic of *Mesocyclops thermo-*

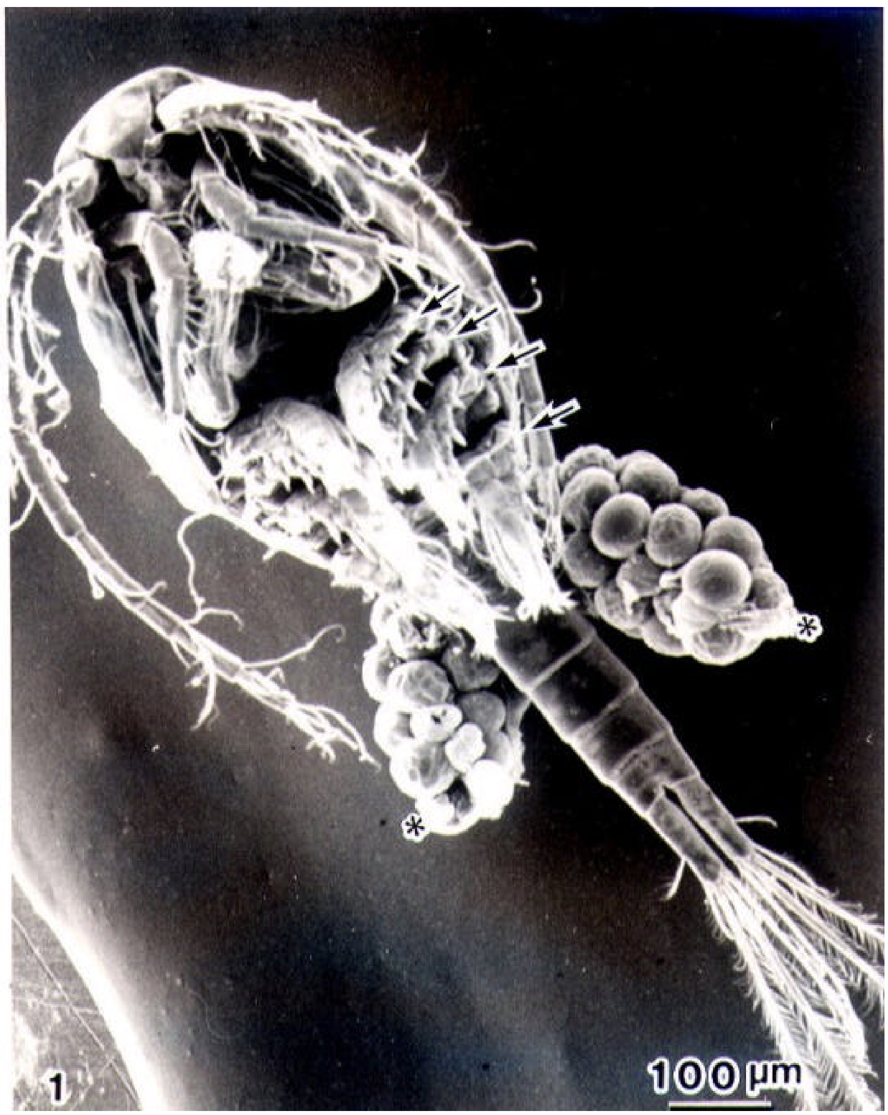

Fig. 1. Panoramic image of scanning electron microscopy of an adult female of *Mesocyclops thermocyclopoides*. The egg bags are indicate by asterisks, and the arrows pointed the 1st to 4th pairs legs. Other structures, such as antenules, antennas, and mouth parts are indicate in figure 2D.

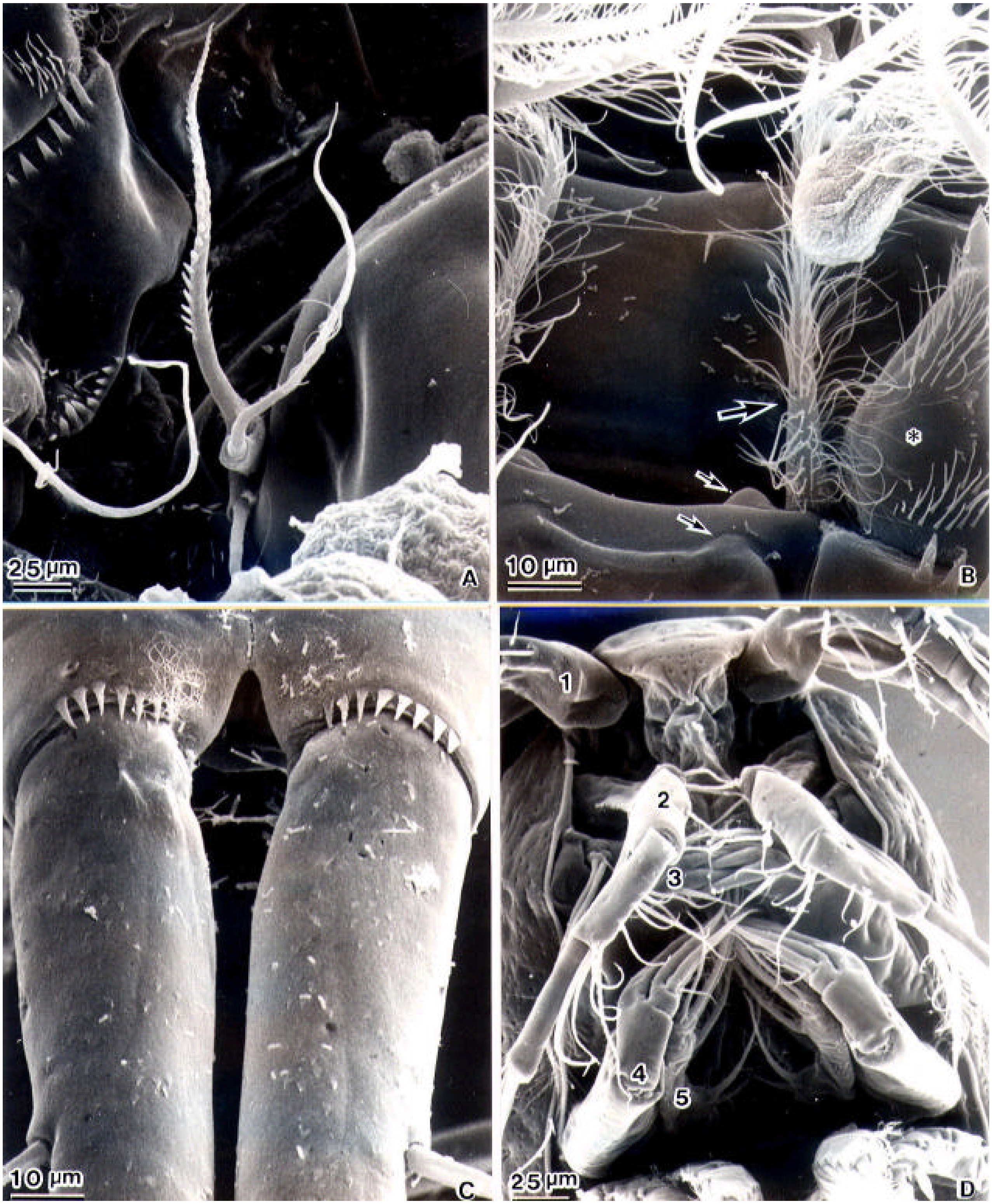

Fig. 2. A, The 5th pair of legs (P5) is characteristic for the genus *Mesocyclops*. The P5 is biarticulate and the spine inserts at the inner margin of the final articulation. Also, the spine is long and thorn-like. B, The ventral plate in-between the 4th pair of legs (asterisk) is distinguishing the genus *Mesocyclops*. Note the setae (arrow) and the tubercles (small arrows) on the plate. C, Furca of *M. Thermocyclopoides*: this species does not show a row of small hairs at the inner margin of the furca, unlike other species of the genus *Mesocyclops*. D, Mouth parts and adjoining structures of *M. thermocyclopoides*. (1) antennule (2) Antenna, (3) Maxillule, (4) Maxilla, (5) Maxilliped, and (6) first pair of legs.

cyclopoides (Fig. 2C). Also, the different mouth parts (mandible, maxilla and maxilipeds) were observed clearly (Fig. 2D).

DISCUSSION

Mesocyclops thermocyclopoides was selected for this study because it is one of the more prevalent species in Costa Rica[1] and previously other authors had used it as a means of biological control in other countries.[7]

Light microscopy identification of the taxonomically important characteristics of *Mesocyclops* always require dissection of the specimens, to remove the abdominal segments and legs. This is a delicate operation that some times damages important structures. In contrast, using SEM allows easy observation of these parts of the specimens without dissection. Nevertheless, a clear disadvantage of SEM is the fact that important structures like the *receptaculum seminis* and the transparent membrane at the antennules could not be seen. Also, the use of t-butyl alcohol method for freeze drying specimens as was modified by Hernández[4] resulted sometimes in scrambled surfaces of the copepods (images not showed). Thus, critical point drying using CO_2 results in better preservation of specimens. Our conclusion is that SEM and light microscopy could be used together to provide good and complementary information on copepods description.

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