Rev. Brasil. Biol., 50(3):799-804
Agosto, 1990 – Rio de Janeiro, RJ

METHOD OF INCLUSION IN PARAFFIN TO OBTAIN SUCCESSIVE SECTIONS OF *BOVICOLA CAPRAE*, GURLT, 1843, (INSECTA: MALLOPHAGA)

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(With 1 figure)

RESUMO

Método de Inclusão em Parafina para Obtenção de *Bovicola caprae*, Gurlt, 1843 (Insecta: Mallophaga) Cortes em Série

No presente trabalho foi ensaiado um método de inclusão de *Bovicola caprae*, Gurlt, 1843, em parafina, usando parafina com dois pontos de fusão diferentes e tratando o material com HCl 1N durante uma hora a 37°C antes da fixação, com o fim de amolecer a cutícula.

Palavras-chave Mallophaga, método de inclusão em parafina, *Bovicola caprae*.

ABSTRACT

In this study we test the method of inclusion in paraffin for *Bovicola caprae* Gurlt, 1843, using paraffin with two different melting points and treating the material with 1N hydrochloric acid for one hour at 37°C, in order to soften the cuticle.

Key words: Mallophaga, method of inclusion in paraffin, *Bovicola caprae*.

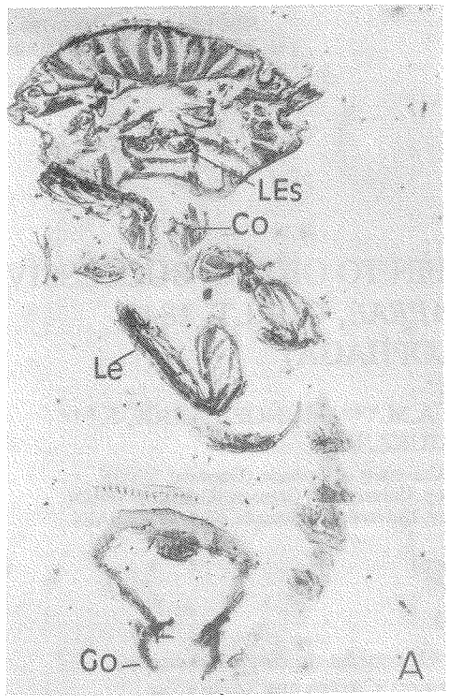
INTRODUCTION

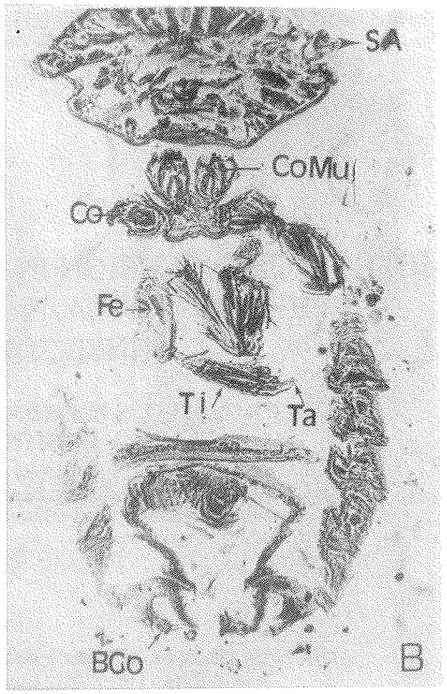
The usual methods of inclusion and fixing have been modified in this series of experiments, due to the fact that Mallophaga are covered with an impermeable cuticle, difficult to penetrate with the usual fixatives.

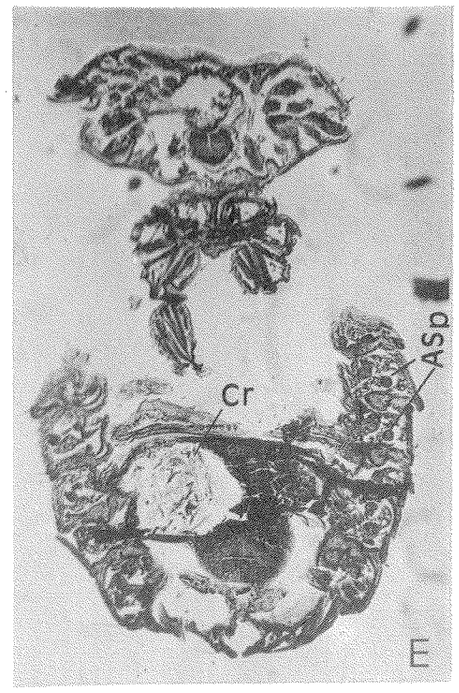
Moreover, we had to control the fixing time exactly, since the material became fragile and brittle with excess time. On the other hand, we had to be cautious as well with dehydration on using, because if prolonged, the tissues grow hard.

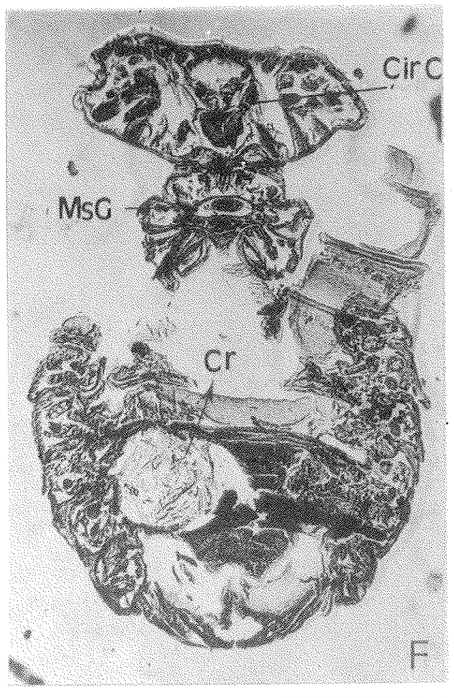
Risler (1951), Symmons (1952), Vijayambika (1974), Radwan and Novak (1977), Saxena and Agarwal (1980), De la Vega y Fernández Marcial (1981) and Srivastava and Agarwal (1983), use for different arthropods such fixatives as Carnoy, Bouin (hot or cold), Zenker, or alcoholic Bouin. They use different physical methods as well, such as heat, agitation, and introduction of fixatives in the tissue with capillary tubules in order to increase penetration.

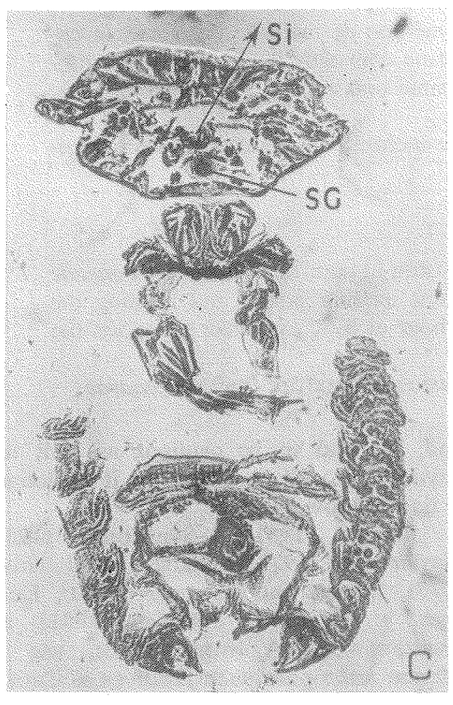
Received August 2, 1985
Accepted December 1, 1989
Distributed August 31, 1990

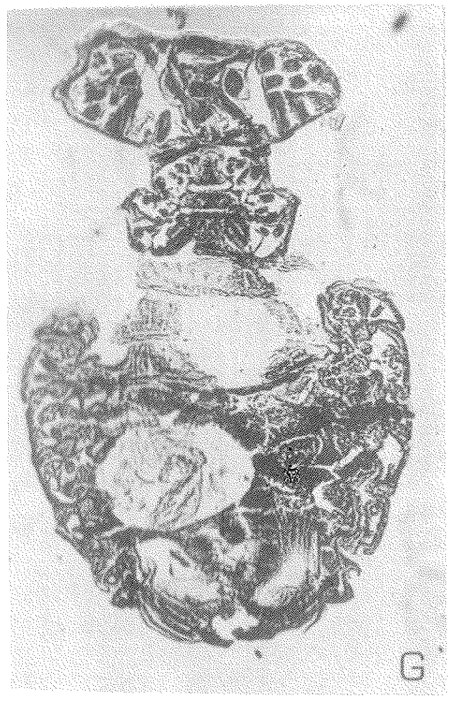












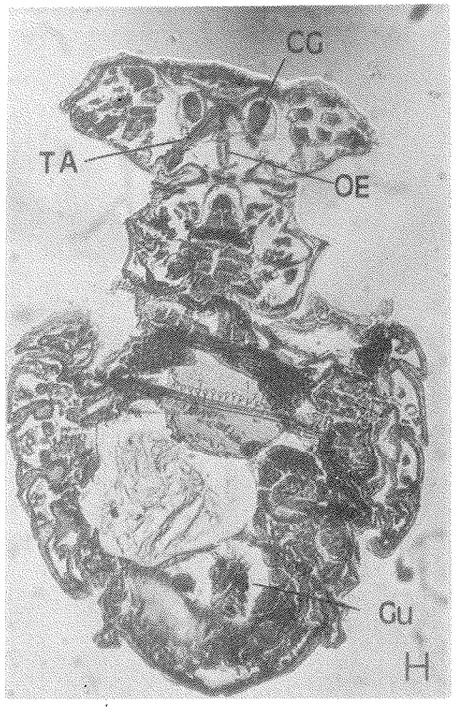
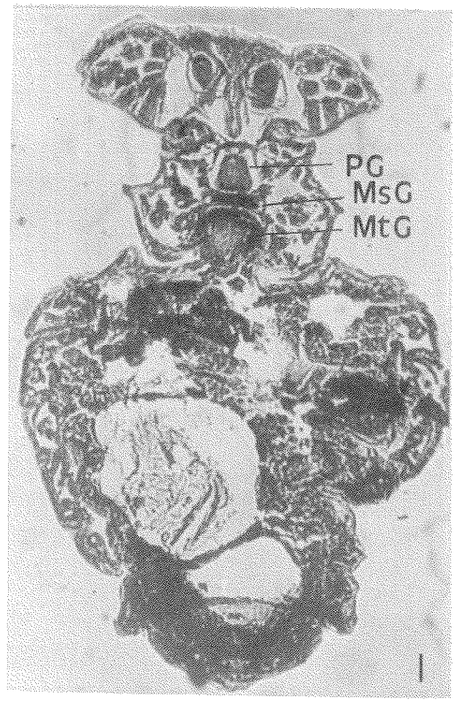
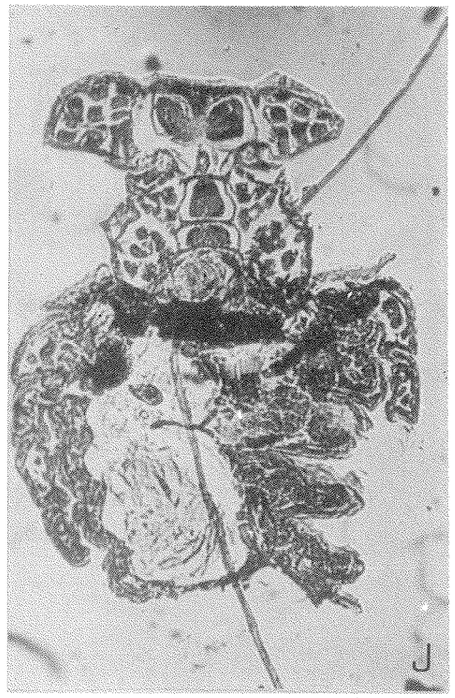
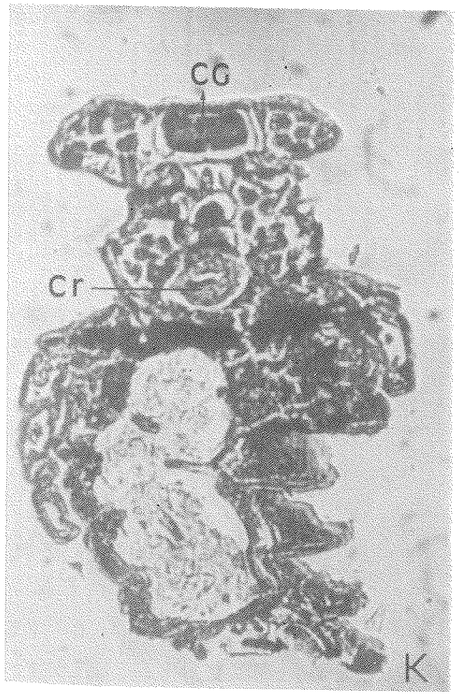


Fig. 1 (A a D) – *Bovicola caprae* Gurlt, 1843; Successive longitudinal sections of head, thorax and abdomen. ASp - abdominal spiracles; BGo - base of gonopod; CG - cerebral ganglion; CirC - circumoe sophageal connective; Co - coxa; CoMu - coxal muscles; Cr - crop; FE - femur; Go - gonopod; Gu - gut; Le - leg; LEs - lingual esclerite, MsG - mesothoracic ganglion, MtG - metathoracic ganglion; OE - oesophagus; PG - prothoracic ganglion; SG - suboesophageal ganglion; Si - sitophore; TA - tentorial arm; Ta - tarso; Ti - tibia.

Fig. 1 (E a H) – *Bovicola caprae* Gurlt, 1843; Successive longitudinal sections of head, thorax and abdomen. ASp - abdominal spiracles; BGo - base of gonopod; CG - cerebral ganglion; CirC - circumoe sophageal connective; Co - coxa; CoMu - coxal muscles; Cr - crop; FE - femur; Go - gonopod; Gu - gut; Le - leg; LEs - lingual esclerite, MsG - mesothoracic ganglion, MtG - metathoracic ganglion; OE - oesophagus; PG - prothoracic ganglion; SG - suboesophageal ganglion; Si - sitophore; TA - tentorial arm; Ta - tarso; Ti - tibia.







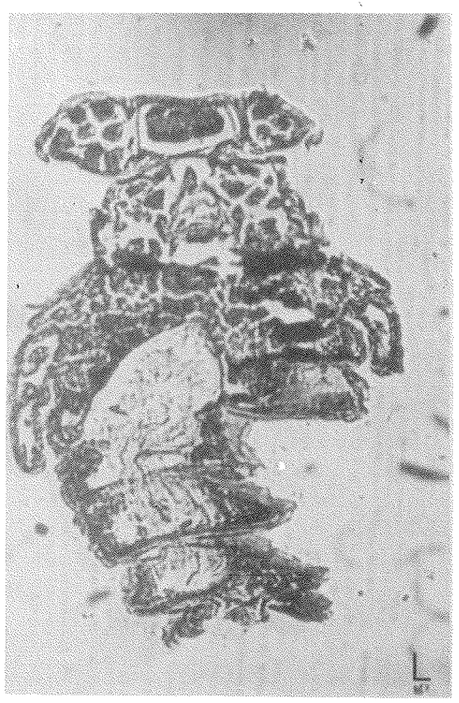


Fig. 1 (I a L) – *Bovicola caprae* Gurlt, 1843; Successive longitudinal sections of head, thorax and abdomen. ASp - abdominal spiracles; BGo - base of gonopod; CG - cerebral ganglion; CirC - circumoe sophageal connective; Co - coxa; CoMu - coxal muscles; Cr - crop; FE - femur; Go - gonopod; Gu - gut; Le - leg; LEs - lingual esclerite, MsG - mesothoracic ganglion, MtG - metathoracic ganglion; OE - oesophagus; PG - prothoracic ganglion; SG - suboesophageal ganglion; Si - sitophore; TA - tentorial arm; Ta - tarso; Ti - tibia.

METERIAL AND METHODS

We studied *Bovicola caprae* females (Gurlt, 1843), which arrived alive and were used immediately for fixing. See Table I.

TABLE I

Method used in *B. caprae* fixing and inclusion

Before the fixing	Hydrochloric acid	1 h, 37°C
Fixation	Alcoholic Bouin	2 h, 37°C
Dehydration	Ethanol 90°	1 5 h
	Ethanol 100°	twice, 6 h each one
Clearing	Methyl benzoate	3 times, 1/2 h each one
	Benzene	1/2 h
Impregnation of paraffin	Benzene-Paraffin 42-44°C	1/2 h
Embedding	Paraffin 43-44°C	1/2 h each one, 3 times
Inclusion	Paraffin 51-53°C	
Deparaffination	Xylene	15 min.
Hydration	Ethanol 100°, 90°, 70° 50°, 30°	1 min. in each one
	destilled water	1 min.
Staining	Hematoxylin (Delafield)	4 min.
	water	10 min.
	eosin	4 min.
	water	1 min.
Differentiate and dehydration	Ethanol 96°, 100°	1 min. in each one
Transparentation	Xylene	1 min.
Mounting.	Eukit	

The material before the fixing keept during one hour at 37°C in hydrochloric acid 1 Normal, in order to soften the cuticle, and we used paraffin with two different melting points for inclusion of the Mallophaga.

We cut the sections lengthwise (thickness 6-10 microns) starting with the ventral part of the Mallophaga, and ending at the dorsum.

RESULTS AND DISCUSSION

Photographs of the histological sections (Fig. 1), obtained with alcoholic Bouin fixing, are presented.

To fix any arthropod and in this case, Mallophaga, one has to penetrats its hard cuticle cover.

Risler, in 1951, made histological sections of *Bovicola caprae*, using fixings such as Carnoy and Bouin. After various experiments, we obtained the best results with alcoholic Bouin and consider it appropriate, particularly for the nervous, digestive and respiratory systems.

In the figures we see the cuticle cover in perfect condition and recognize also the longitudinal and transverse bundle shaped muscular tissues. The nervous system, as well as the cerebral ganglions and the three thoracic ganglions, are clearly visible in Fig. 1. In relation to the digestive system, we see in Fig. 1-H the intestinal contents, in Fig. 1-K the crop and in Fig. 1-H, the oesophagus.

Concerning the respiratory system we observe two spiracles in each abdominal segment, as shown in Fig. 1-D.

Symmons (1952) studied the anatomy of the Mallophagan head, using hot Bouin and Zenker fixings while, the inclusion was done with celoidine. The method of inclusion in paraffin is less difficult. We used paraffin at 42-44°C for the impregnation of the specimens because this melting point is appropiate for the inclusion of the delicate material, since a higher temperature could damage the tissues. For the definitive inclusion and finishing, we used paraffin with a melting point of 51-53°C. Using these two waxes with different melting points, we obtained always homogenous inclusions.

Vijayambika (1974) and Saxena and Agarwal (1980) used alcoholic Bouin's for fixing mites and *Lipeurus lawrensis tropicalis* Peters, but did not use the previous step of introducing the specimens in 1 Normal hydrochloric acid during one hour at 37°C.

This makes penetration of the fixative easier through the Mallophga's chitin cover.

We consulted also de la Vega and Fernandez Marcial (1980), who managed to minimize the process of inclusion in paraffin, using heat and agitation. These authors increased the temperature of specimens at every step of the fixing and inclusion process.

Acknowledgements - We thank Dr. Illescas for her assistence in carrying out the photographs of this work.

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