

Scientific note

A scientific note on the prevalence of trypanosomatid parasites of honey bees (*Apis mellifera* L.) in southern Spain

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Little research has been carried out on the flagellate protozoans that affect the honey bee (*Apis mellifera* L.). As a result, biological cycles and pathogenicity of these protozoans for the honey bee are not well known. Flagellate infection is quite common in spring and summer but in winter infection levels are hardly recorded [1, 3, 4]. The reasons for this are not clear, but climatic and nutritional factors could play some part [8].

Flagellates have commonly been found in Europe and South Africa [10], including *Leptomonas apis* and *Crithidia* sp. Langridge and McGhee [7] managed to cultivate organism isolates in apiaries in Victoria (Australia) identified as *Crithidia mellificae*. Many of these protozoans are probably not specific to the honey bee and may be the same as those that affect other insects [10].

In the intestines of the bumble bee (*Bombus* sp.) the flagellate trypanosomatids *Crithidia bombi* and *Leptomonas* sp. are well reported [5, 8, 9].

The object of this study was to investigate the prevalence of the trypanosomatid parasites of bees in apiaries in southern Spain.

Samples were collected in 12 experimental apiaries situated in the south of the Iberian Peninsula between October 1990 and October 1992. Thirty-five Layens type hives were sampled during this period at different times of year. One hundred and ninety samples (worker bees) were collected from the hives and conserved in 70 %

ethanol pending their analysis. Adult worker bees, of unknown age, were collected from the brood nest, from mixed brood nests and honey storage areas and from the hive entrance.

Each sample was made up of 60 bees, which were analysed individually, homogenising their intestine in a eppendorf tube with the help of a small piston. Extensions were prepared from the positive samples which were fixed for 2 min with methanol and then stained with Giemsa at 0.25 % for 12–18 h. Microscopic examinations were made at 400× or 1 000×.

The geographical distribution of the sampled stations was Cáceres, Castellar de la Frontera (Cádiz), Dos Hermanas (Sevilla), Hornachuelos (Córdoba), Lepe (Huelva), Trassiera (Córdoba), Lanjarón (Granada), Vadillo-Cazorla (Jaén), Maro-Nerja (Málaga), Pinos del Valle (Granada), Berja-Laujar (Almería) and Murcia.

Of the 12 apiaries studied, trypanosomatid parasites (figure 1) were detected in three apiaries (25 %), in two hives in Lanjarón (Granada), one hive in Maro-Nerja (Málaga) and one in Cáceres, representing 11.4 % ($n = 35$) of the hives studied. However, very few samples and bees were infected, 2 % ($n = 199$) and 0.12 % ($n = 11\ 940$), respectively. The infection in the four positive samples ranged between 1.7 and 11.8 % ($\bar{x} = 6.2 \% \pm 4.6 \%$), and appeared in the months of July and August.

The samples of Cáceres were collected in August 1991 with an infection of 1.7 %. The samples of Lanjarón (Granada) were collected

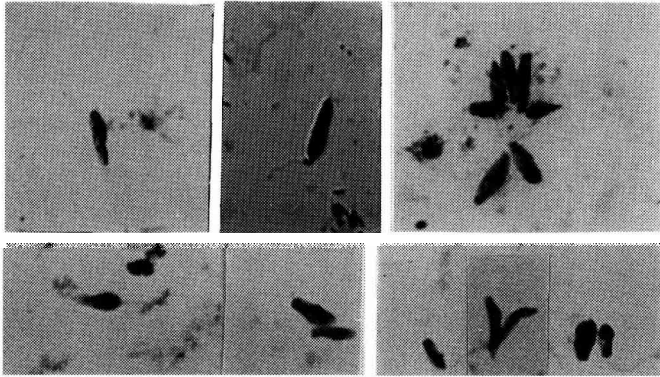


Figure 1. Photographs of diverse trypanosomatids parasites of honey bee found in this study ($\times 1\,000$).

from two hives on July 1991 with parasitism rates of 11.8 % and 8.3 %. The sample from Maro-Nerja (Málaga) was collected on July 1991 with a parasitism rate of 3.3 %.

The morphology of these trypanosomatids was as follows: Choanomastigotes: body-length 9–16 mm ($\bar{x} = 11.6 \pm 1.8$ mm; $n = 27$), width 3–5 mm ($\bar{x} = 3.6 \pm 0.5$ mm; $n = 27$), flagellum 9–18 mm ($\bar{x} = 13.6 \pm 4$ mm; $n = 5$). Amastigotes: length 7–8 mm ($\bar{x} = 7.6 \pm 0.5$ mm; $n = 11$), width 2–4 mm ($\bar{x} = 3.1 \pm 0.6$ mm; $n = 11$). The relative uniformity in the morphology of the Family Trypanosomatidae, together with the means of conserving the samples (ethanol at 70 %, making them useless for isoenzymatic studies) made it impossible for us to assign any taxonomic position.

There is no evidence that the flagellates are pathogenic [2]. However, the pathogenic action of these trypanosomatid parasites must be reviewed. Landridge [6] found species of the genus *Crithidia* in bees to have caused a high degree of mortality, in spite of the fact that these species are not pathogenic. In this study, in some of the counts made following the routine methodology for counting *Nosema apis* spores, up to 1.6×10^6 flagellates were recorded in the intestine of some bees.

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Note scientifique sur la fréquence de parasites Trypanosomatidés de l'abeille (*Apis mellifera* L.) dans le sud de l'Espagne

Wissenschaftliche Notiz über die weite Verbreitung von trypanosomen Parasiten bei Honigbienen (*Apis mellifera* L.) in Süds Spanien.

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