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Note

A newly recorded neogregarine (Protozoa, Apicomplexa), parasite in honey bees *(Apis mellifera)* and bumble bees (*Bombus* spp)

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Summary — A new infectious disease of *Apis mellifera*, *Bombus hortorum* and *B terrestris* is reported to be caused by a parasitic protozoan belonging to the order Neogregarinida. The disease can be diagnosed based on the presence of characteristic navicular spores measuring 11.4–14.4 x 3.6–5.4 μm. Infection level in host populations was low but since this parasite was found in host insects in Finland and Italy, it evidently has a wide distribution in Europe.

***Apis* / *Bombus* / neogregarine / protozoan / parasite**

While continuing our studies (Lipa and Triggiani, 1988; Triggiani, 1991) on spiroplasma, flagellate and microsporidian infections of the honey bee (*Apis mellifera* L) and bumble bees (*Bombus* spp) in some adults collected in Italy and in Finland very peculiar spores were observed which could not be attributed to known pathogens of these beneficial insects. A preliminary report is therefore presented to alert researchers in various countries as to the occurrence and incidence of the pathogen involved.

The new pathogen was first noticed in 1988 in flying workers and queens of *Bombus hortorum* L and *B terrestris* L collected by sweep net from various crops in Apuglia (southern Italy). When the same pathogen was also recorded in 1990 in one *A mellifera* worker from a hive in the experimental apiary at the Agricultural Research Center in Jokionen, Finland, it was concluded that the pathogen was a new parasitic protozoan which, based on the type of spores and the life cycle, belongs to the order Neogregarinida.

The detailed data on host, country and locality records in which the new pathogen was noted are given in table I. These data indicate that the infection level by this neogregarine is very low, which is not surprising as infections of the honey bee *(A mellifera)* by eugregarines (Stejskal, 1965) or flagellates (Langridge, 1966) are also generally at a very low level and have been reported from very few localities. However,

Table I. Host and localities in which a new neogregarine infection was observed.

Host and locality	*No of insects examined*	*No of insects infected*
Bombus hortorum		
Carpino, Italy; 2 May 1988	17	1[1]
Massafra, Italy; 23 May 1988	3	1[1]
Bombus terrestris		
Lesina, Italy; 17 Feb 1988	33	1[1]
Policoro, Italy; 20 Feb 1988	34	1[2]
Massafra, Italy; 23 Feb 1988	30	2[1]
Apis mellifera		
Jokioinen, Finland; 23 July 1990	34	1[1]

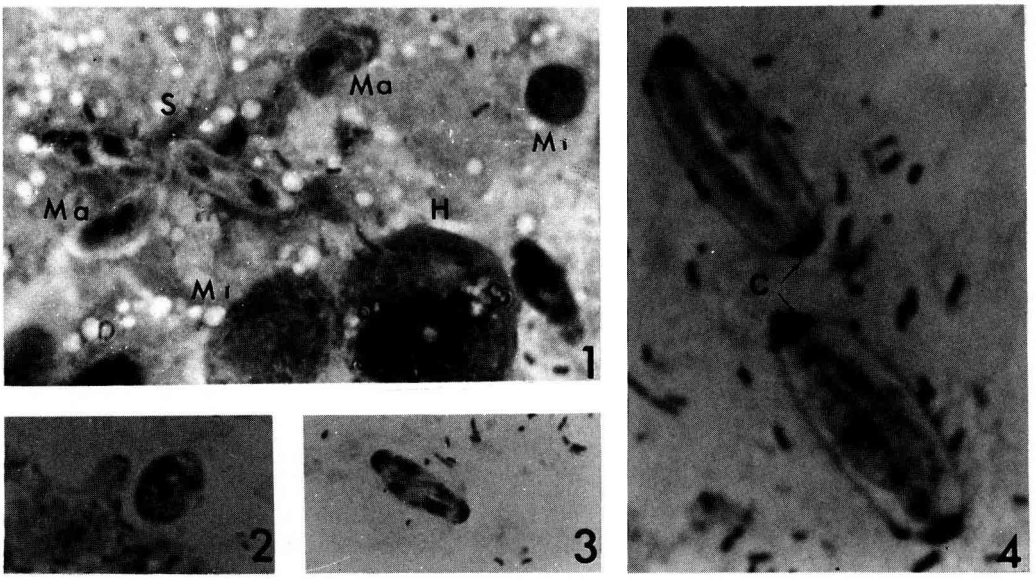
[1] Workers; [2] queens.

since this parasitic neogregarine was found in Finland and Italy we assume that it has a wide distribution in Europe and that a thorough survey may indicate that it also occurs in other countries or continents.

The following diagnostic features may be helpful in survey and identification of the pathogen involved. On smeared fat body preparations of *A mellifera* and *Bombus* spp, various developmental stages were observed. Micronuclear meronts contain up to 40 nuclei and have a diameter of up to 45 μm (fig 1). Micronuclear merozoites are oval or elongated in shape and measure 5–9 x 2.5–3.5 μm (fig 1).

Macronuclear meronts measure 20 x 15 μm and give rise to ovoid or pyriform macronuclear merozoites 5–8 μm in diameter (figs 1,2).

The spores observed in *Apis* and *Bombus* were identical. They are navicular in



Figs 1–4. Developmental stages of a parasitic neogregarine. **1.** Spores (S), micronuclear (Mi) meronts, macronuclear (Ma) merozoites (Ma), and honey bee (A mellifera) hemocyte (H); x523. **2.** Binucleate macronuclear merozoite in smeared tissues of *B terrestris*; x435. **3.** Young spore with 4 visible uninucleate sporozoites; x348. **4.** Two mature spores from *A mellifera*, with clearly visible polar caps (C) and enigmatic sporozoites; x1800.

shape, and have distinct caps (figs 3,4). Spores fixed in methanol for 2 min and stained with 0.25% Giemsa for 15 h are 11.1–14.4 μm long and 3.6–5.4 μm wide. Inside the stained young spores 4 uninucleate sporozoites are readily visible (fig 3); however, they become less visible in the mature spores (fig 4).

The life cycle and morphology of meronts and spores indicate that the protozoan infecting *A mellifera* and *Bombus* spp belongs to the order Neogregarinida (= Schizogregarinida) (Apicomplexa). Its spores (sporocysts) differ greatly from those of presently known genera as they contain only 4 sporozoites while spores of the genera *eg Mattesia*, *Farinocystis* or *Syncystis* contain 8 sporozoites. In addition, the spores of this newly reported neogregarine are narrow and have a delicate wall. All these features indicate that this neogregarine constitutes both a new species and a new genus. It also represents the first record of neogregarine infection in the Apidae.

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Résumé — Un nouveau parasite néogrégarine (Protozoa, Apicomplexa) de l'abeille *(Apis mellifera)* et du bourdon (*Bombus* sp) (Hymenoptera, Apidae). L'examen au microscope de tissus d'adultes d'*Apis mellifera*, récoltés en 1990 en Finlande, et de *Bombus terrestris* et de *B hortorum*, récoltés en 1988 en Italie, a montré la présence de spores d'un protozoaire encore inconnu. D'après le cycle de développement du parasite, étudié sur des frottis du corps gras fixés 2 min au méthanol et colorés avec une solution de Giemsa à 0,25% durant 8–12 h, le parasite appartient à l'ordre des Neogregarinida. Les mérontes macronucléaires renferment jusqu'à 40 noyaux, leur diamètre atteint 45 μm. Ils mesurent 20 x 15 μm et donnent naissance à des mérozoïtes macronucléaires (ma) ovoïdes ou pyriformes ayant un diamètre de 5–8 μm (figs 1–2). Les mérozoïtes micronucléaires sont de forme ovale ou allongée et mesurent 5-9 x 2,5–3,5 μm (fig 1). Les spores observées chez *Apis* et chez *Bombus* étaient identiques. Elles sont naviculaires et possèdent des chapeaux distincts aux 2 extrémités. Les spores fixées et colorées ont 11,1–14,4 μm de long et 3,6–5,4 μm de large. À l'intérieur des spores colorées on peut facilement reconnaître 4 sporozoïtes contenant chacun un noyau (fig 3). La morphologie des premiers stades de développement et les caractéristiques morphologiques des spores différencient ce nouveau néogrégarine des genres *Mattesia* et *Farinocystis.* Nous considérons donc que le néogregarine découvert chez *Apis* et *Bombus* est un genre nouveau et une espèce nouvelle. Comme le montre le tableau I, le niveau d'infestation dans les colonies d'abeilles et de bourdons étudiées était relativement faible. Néanmoins l'intensité de l'infestation chez certains individus parasités était très forte. Ceci prouve que le pathogène peut diminuer les processus vitaux et les activités des hôtes parasités. Sa répartition géographique est étendue puisqu'on l'a trouvé aussi bien en Finlande qu'en Italie.

Zusammenfassung — Ein neu beschriebenes neogregarines parasitisches Protozoon bei *Apis mellifera* und *Bombus* sp. Die mikroskopische Untersuchungen von 1990 in Finnland gesammelten *A mellifera*-Arbeiterinnen und von *Bombus terrestris* und *B hortorum*, die 1988 in Italien ge-

sammelt worden waren, ergab den Befund von Sporen eines bisher unbekannten parasitischen Einzellers. Der Lebenszyklus des Parasiten, untersucht an Ausstrichen des Fettkörpers (2 min fixiert in Methanol und gefärbt in 0.25% Giemsa-Lösung für 8-12 h) bewies, daß er zur Ordnung der Neogregarinida gehört. Macronucleäre Meronten enthalten bis zu 40 Nuclei und besitzen einen Durchmesser von bis zu 45 µm. Micronucleäre Merozyten sind oval oder länglich und messen 5-9 x 2.5-3.5 µm (Abb 1). Macronucleäre Meronten messen 20 x 15 µm und erzeugen ovoidale oder birnenförmige macronucleäre Merozyten (ma) mit einem Durchmesser von 5-8 µm (Abb 1-2).

Die bei *Apis* und *Bombus* beobachteten Sporen (Oozysten) waren gleich. Sie haben die Form eines Bootes und tragen an beiden Enden deutliche Kappen. Fixierte und gefärbte Sporen sind 11.1–14.4 µm lang und 3.6–5.4 µm breit. Im Inneren der gefärbten Sporen sind deutlich vier Sporozoiten mit je einem einzigen Kern zu sehen (Abb 3).

Die Morphologie der frühen Entwicklungsstadien und morphologische Merkmale der Sporen unterscheiden diese neu beschriebene Protozoe von den Gattungen *Mattesia* und *Farinocystis.* Deshalb betrachten wir die von uns bei *Apis* und *Bombus* neu beschriebene Neogregarine als neue Gattung und neue Art.

Wie aus Tabelle I zu ersehen, ist der Infektionsgrad bei den untersuchten Bienenvölkern und Hummelkolonien relativ niedrig. Dennoch war die Intensität der Infektion bei einigen erkrankten Tieren ziemlich hoch und dies beweist, daß der Erreger die Lebensvorgänge und die Aktivitäten der befallenen Wirte beeinträchtigen kann. Der Erreger hat offensichtlich eine weite geographische Verbreitung, da er sowohl in Finland wie in Italien beobachtet wurde.

Parasitologie / Protozoa / Neogregarinida / *Apis* / *Bombus*

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