Notas

First record of *Apicystis* sp. (Apicomplexa: Neogregarinorida) in Moroccan honey bees

Primer registro de Apicystis sp. (Apicomplexa: Neogregarinorida) en abejas melíferas marroquíes

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Abstract: Pollinator Hymenoptera, particularly those of the Apidae family, are currently -to a greater or lesser extent- threatened. Several abiotic factors such as an intensive use of pesticides, modernization of agricultural practices or habitat degradation as well as biotic factors such as associated pathogens, have been reported to be responsible for bee colony collapses worldwide. Certain pathogens, consisting of unicellular eukaryotic organisms, belonging to few genera have been recognized as commonly associated to hymenopteran hosts. Among these pathogens, we can find *Vairimorpha* (*Nosema*) Nägeli (Microsporidia), *Crithidia* / *Lotmaria* (Protista: Euglenozoa), *Malpighamoeba* Prell (Protista: Amoebozoa) and *Apicystis* Lipa & Triggiani (Protista: Apicomplexa). One species of the later, *Apicystis bombi* Liu et al., is mainly found associated to bumble bees (*Bombus* spp.), but can occur in the honey bee *Apis mellifera* L. with its effects not fully understood. The current note reports a finding of *Apicystis* infections in workers of two native honey bee subspecies (*A. mellifera* intermissa von Buttel-Reepen and *A. mellifera sahariensis* (Baldensperger)) during a health survey in apiaries from Souss-Massa, central Morocco, Africa. This record updates the epizootiological data, both regionally and worldwide.
Keywords: *Apis mellifera intermissa*, *Apis mellifera sahariensis*, Emerging disease, Maghreb, Pollinator.
Resumen: Los himenópteros polinizadores, en particular aquellos de la familia Apidae, se encuentran actualmente -en mayor o menor medida- amenazados. Varios factores, tanto abióticos (uso intensivo de pesticidas, modernización de las prácticas agrícolas, degradación del hábitat) como bióticos (parásitos, patógenos), parecen estar implicados en el colapso de las colonias de abejas en todo el mundo. En lo que a patógenos respecta, se han reconocido organismos eucariotas unicelulares pertenecientes a unos pocos géneros que están comúnmente asociados a himenópteros polinizadores. Entre dichos patógenos se encuentan: *Vairimorpha* (*Nosema*) Nägeli (Microsporidia), *Crithidia* / *Lotmaria* (Protista: Euglenozoa), *Malpighamoeba* Prell (Protista: Amoebozoa) y *Apicystis* Lipa & Triggiani (Protista: Apicomplexa). Una especie de este último, *Apicystis bombi* Liu et al., se encuentra principalmente ligada a abejorros (*Bombus* spp.), aunque ocasionalmente se ha detectado en la abeja melífera *Apis mellifera* L. sin efectos completamente conocidos sobre este hospedador. En la presente nota se informa el hallazgo de infecciones por *Apicystis* en obreras de dos subespecies nativas de abejas melíferas (*A. mellifera* intermissa von Buttel-Reepen y *A. mellifera* sahariensis (Baldensperger)) durante un estudio sanitario en colmenares de Souss-Massa, región central de Marruecos, África. Este registro actualiza los datos epizootiológicos, tanto a nivel regional como mundial.

Palabras clave: *Apis mellifera intermissa*, *Apis mellifera sahariensis*, Enfermedad emergente, Magreb, Polinizador.

Hymenopteran pollinators, such as honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) (Apidae), are key species in the process of angiosperm pollination, ensuring the well-being of ecosystems, the preservation of biodiversity, and food production (Gallai et al., 2009; Schulp et al., 2014; Mouillard-Lample et al., 2023). The population dynamics of these insects is permanently threatened by several, external factors, of which entomopathogens are key. These infectious agents are mainly found in honey bees sampled in commercial apiaries, but not in bumble bees or other bees worldwide, where understanding of parasitic diseases is still in its early stages (Goulson et al., 2015; Proesmans et al., 2021).

Honey bees can be affected, besides viral and bacterial agents (Pasho et al., 2021), by other unicellular parasites such as Microsporidia (*Vairimorpha apis* (Zander) and *V. ceranae*[1]Fries et al.) and protists [*Crithidia mellificae* (Langridge & McGhee), *Lotmaria passim* Schwarz (Euglenozoa) and *Malphigamoeba mellificae* (Prell) (Amoebozoa)]. Both *V. apis* and *V. ceranae* cause the disease called Nosemosis (Mazur & Gajda, 2022; Parrella et al., 2024), whereas the protists which may (or not) occur with a relevant health impact (Schwarz et al., 2015; Shäffer et al., 2022). In the last 10-15 years, another protozoan that normally infects bumble bees, the neogregarine *Apicystis bombi* Liu et al. (Apicomplexa: Neogregarinorida) was registered in honey bees from South America, North America, Europe, Asia and Africa (reviewed by Plischuk & Lange, 2024). More recently, a new species of *Apicystis* Lipa & Triggiani (*A. cryptica* Schoonvaere et al.) was isolated from *Bombus pascuorum* Scopoli in Belgium, being this bumble bee, its only known host so far (Schoonvaere et al., 2020). Both *Apicystis* species have been detected in the adipose tissue of their hosts, where they disrupt tissue integrity and lead to subsequent nutritional imbalances. The extent to which *A. bombi* displays comparable virulence in other hosts - such as honey bees or carpenter bees, in which it was also found - remains unclear (Chantawannakul et al., 2016; Plischuk et al., 2023; Plischuk & Lange, 2024 and references therein).

(1)Formerly Nosema apis and Nosema ceranae, resp. (Tokarev et al., 2020 but also see Bartolomé et al., 2024).

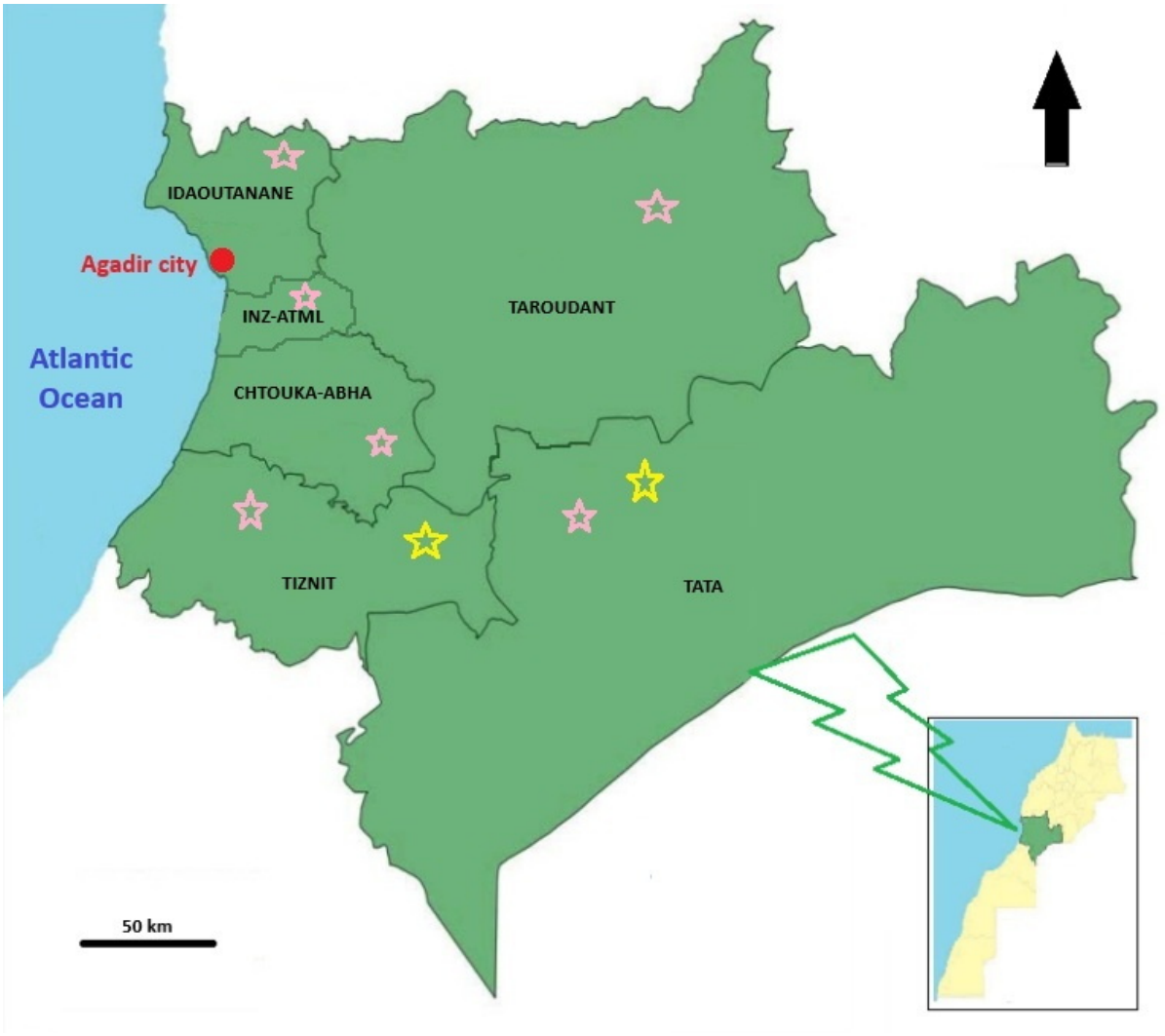

Figure 1.

Souss-Massa region, Morocco.

Yellow stars indicate *Apicystis-positive A. melliferasahariensis* apiaries. Pink stars indicate *Apicystis*-positive *A. melliferaintermissa* apiaries.

In this research, 206 samples of adult honey bee workers were collected from 206 apiaries across the Souss-Massa region, southwestern Morocco (30°5'13"N; 8°27'38"W; Fig. 1). Each sample consisted of a pool of ~80 bees taken from the same apiary. Bees were identified and preserved in 70 % ethanol flasks until analyses. Note that the two subspecies sampled are easily distinguishable: *A. mellifera sahariensis* (Baldensperger) ("Saharan honey bee") is more yellowish than *A. mellifera intermissa* von Buttel-Reepen("Tellian honey bee"). The analyses were conducted using a microscopic screening of homogenized bee metasomes (PCM, 40x – 100x). Morphological characters described by Lipa & Triggiani (1996), and Plischuk & Lange (2009) were used to recognize the *Apicystis* oocysts (Fig. 2). Presence of *Apicystis* was observed in 17 samples, meaning a total prevalence of 8.25 % in that study area. The cases concerned five

samples of *A. mellifera sahariensis* (n= 39) and twelve samples of *A. mellifera intermissa* (n= 167).

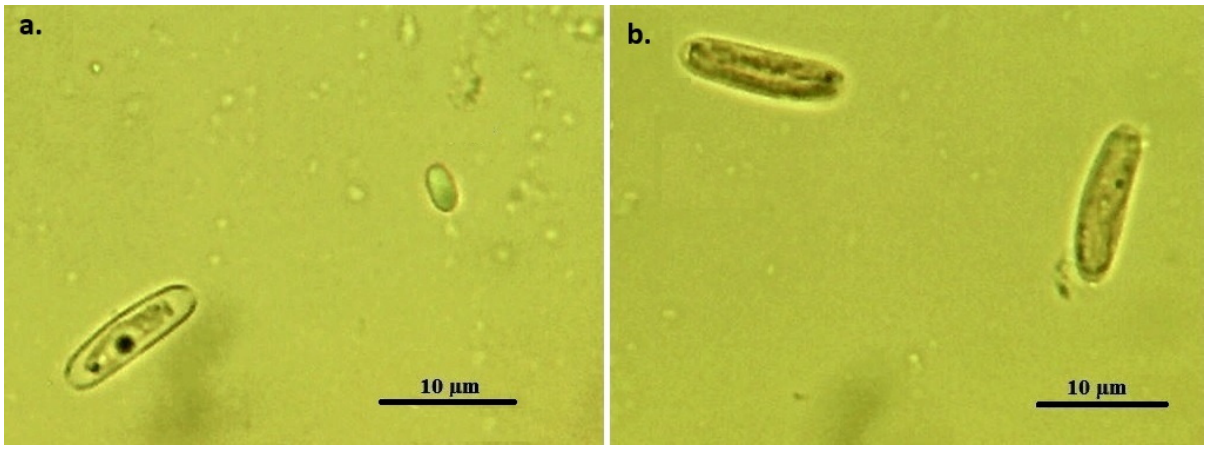

Figure 2.

***Apicystis* sp. oocysts.**

a. Mature oocyst (left) and a spore of *Varimorpha* sp. (right). b. Oocysts showing sporozoites inside.

These findings allow us to report the occurrence of *Apicystis* sp. in Moroccan honey bees for the first time. Furthermore, this record constitutes the second report from Africa after that of Menail et al. (2016), who reported the presence of *A. bombi* in *A. mellifera intermissa* adults (prevalence= 2.27 %; n= 44) collected in the Annaba region, Algeria (*ca*. 1,600 km from Souss-Massa region).

This parasite appears to infect bumble bees as its natural hosts; however, in the last decade this apparent specificity has been expanded and it should not be ruled out that it could also be harmful in certain alternative hosts such as honey bees or carpenter bees (*Xylocopa* spp.), although the pathogenicity of *Apicystis* in these non-*Bombus* species has not been confirmed (Plischuk et al., 2023; Plischuk & Lange, 2024). In this case, unlike what was reported by Plischuk et al. (2011), a large percentage of the observed oocysts using phase-contrast microscopy showed refringence, a fact that could indicate viability. In Poland, Schulz et al. (2019) found oocysts in both hemocoel and ventriculus of *A. mellifera* workers, but not in the adipose tissue. Unfortunately, since Moroccan bees were analyzed by homogenization, the tissue in which these oocysts were lodged could not be determined.

As in South America (see Plischuk & Lange, 2024), the presence of this neogregarine in Souss-Massa, geographically far from other reports, is another interesting case to analyze. Considering that three (or four) species of *Bombus* [*B. lapidarius* (L.), *B. ruderatus* (Fabricius), *B. terrestris* (L.), and probably *B. mocsaryi* Kriechbaumer (Rasmont & Iserbyt, 2010-2013)] have been recorded in Morocco, *Apicystis* could be also parasitizing individuals of these species, or another pollinator species, but reports of this are absent in the entire

region. This could be due to the lack of additional surveys conducted throughout the Maghreb region looking for possible hosts, that could complement the findings of Menail et al. (2016) in Northeastern Algeria and the present report. Given this lack of information, the implementation of monitoring programs to establish the occurrence of both pathogens and parasites in pollinators, remains fundamental and should be initiated as soon as possible in order to protect native bee populations.

Regarding *A. cryptica*, even though it has only been found in bumble bees from Belgium, both *Apicystis* species mentioned are almost indistinguishable based on their morphological characters; therefore, the present work should be furthered using molecular techniques in order to accurately identify the species present in Morocco. However, considering the possible impacts of these pathogens on honey bee health, the present findings are relevant and need to be communicated. Apiculture in the Maghreb region, particularly in Morocco, which ranks the highest in biodiversity across the Mediterranean basin, plays a vital role not only from an ecological point of view but also from a food production, and therefore requires significant attention. In this context, beekeepers should consider potential pathogens, including new emerging diseases that could threat this key sector, not only in the Morocco, but also in similar ecoregions on both shores of the Mediterranean: North Africa and Europe. Authors hope that this report adds another piece in the puzzle of honey bee health-related data worldwide and be considered for further regional research.

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