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Occurrence and distribution of the microsporidium *Vairimorpha* (*Nosema*) spp. in apiaries in Brazil - Systematic review

Ocorrência e distribuição do microsporídio *Vairimorpha* (*Nosema*) spp. em apiários do Brasil - Revisão sistemática

Ocurrencia y distribución de microsporidios *Vairimorpha* (*Nosema*) spp. en colmenares de Brasil - Revisión sistemática

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Abstract
Nosemosis is one of the main diseases of bees, caused by microsporidia of the genus *Vairimorpha* (*Nosema*). The main etiologic agents are *Vairimorpha apis* and *Vairimorpha ceranae*. These pathogens are widely distributed around the world, and in Brazil, they have already been reported in some states, but these data have never been gathered in a review. The objective of this study was to perform a systematic review of the occurrence and distribution of the microsporidium *Vairimorpha* spp. in apiaries in Brazil. The search was done in the SciELO, PubMed, DOAJ, and Capes Journals (Scopus) databases. 14 articles were selected and published in English and Portuguese. In the included publications, only eight Brazilian states were referred (Bahia, Espírito Santo, Goiás, Minas Gerais, Piauí, Rio Grande do Sul, Santa Catarina, and São Paulo). No work was conducted in the northern of the country, and most of the publications are concentrated in the southern and southeastern. Regarding the *Vairimorpha* species, only *V. apis* and *V. ceranae* were investigated, and *V. ceranae* was predominantly detected. Most of the studies were done only with the Africanized bee *Apis mellifera*. This review showed which Brazilian regions have never been explored in terms of incidence and distribution of these pathogens, revealing a gap to be filled by the country's beekeeping surveillance and health systems. In addition, because of the great Brazilian biodiversity, it is evident that the occurrence and distribution of these pathogens also need to be investigated with other bee species.
Keywords: Bees; Apiculture; Microsporidia; Nosemosis; Beekeeping health.

Resumo
A nosemose é uma das principais doenças das abelhas, ocasionada por microsporídios do gênero *Vairimorpha* (*Nosema*). Os principais agentes etiológicos são *Vairimorpha apis* e *Vairimorpha ceranae.* Estes patógenos estão amplamente distribuídos no mundo e, no Brasil, já foram notificados em alguns estados, mas estes dados nunca foram reunidos em uma revisão. O objetivo deste trabalho foi realizar uma revisão sistemática da literatura acerca da ocorrência e distribuição do microsporídio *Vairimorpha* spp. em apiários do Brasil. A busca foi realizada nas bases de dados SciELO, PubMed, DOAJ e Periódicos Capes (Scopus). Foram selecionados 14 artigos, publicados nos idiomas inglês e português. Nas publicações incluídas, apenas oito estados brasileiros foram contemplados (Bahia, Espírito Santo, Goiás, Minas Gerais, Piauí, Rio Grande do Sul, Santa Catarina e São Paulo). Nenhum trabalho foi conduzido

na região norte do país e a maioria destes foi concentrada nas regiões sul e sudeste. Em relação as espécies de *Vairimorpha*, apenas *V. apis* e *V. ceranae* foram investigadas, sendo que, *V. ceranae* foi prevalentemente detectada. A maioria dos trabalhos foram realizados apenas com a abelha africanizada *Apis mellifera*, com exceção de quatro artigos que foram com outras espécies. Essa revisão evidenciou quais as regiões brasileiras que nunca foram exploradas quanto a incidência e distribuição destes patógenos, revelando uma lacuna a ser preenchida pelos sistemas de vigilância e sanidade apícola do país. Além disso, tendo em vista a grande biodiversidade brasileira, fica evidente que a ocorrência e distribuição destes patógenos necessitam também ser investigadas em outras espécies de abelhas.
Palavras-chave: Abelhas; Apicultura; Microsporidia; Nosemose; Sanidade apícola.

Resumen
La nosemosis es una de las principales enfermedades de las abejas, causada por microsporidios del género *Vairimorpha* (*Nosema*). Los principales agentes etiológicos son *Vairimorpha apis* y *Vairimorpha ceranae*. Estos patógenos están distribuidos en el mundo y, en Brasil, ya han sido reportados en algunos estados, pero estos datos nunca han sido recogidos en una revisión. El objetivo de este estudio ha sido realizar una revisión sistemática de la literatura sobre la ocurrencia y distribución de microsporidiales *Vairimorpha* spp. en colmenares de Brasil. La búsqueda se realizó en los archivos SciELO, PubMed, DOAJ y Revistas de la Capes (Scopus). Se seleccionaron 14 artículos, publicados en inglés y portugués. Registros de espécies do género *Vairimorpha* han sido descritos en ocho de los estados brasileños: Bahía, Espírito Santo, Goiás, Minas Gerais, Piauí, Rio Grande do Sul, Santa Catarina y São Paulo. No existen registros de trabajos hechos en la región norte del Brasil. La mayoría de los trabajos realizados fueron desarrollados en las regiones Sur y Sudeste. En estos trabajos, solo se ha investigado las especies *V. apis* y, predominantemente, *V. ceranae*. La mayoría de los estudios se llevaron a cabo con *Apis mellifera*. Esta revisión muestra la demanda de estudios respecto la ocurrencia y distribución de los agentes de la nosemosis em diferentes regiones de Brasil, información necesaria para una mayor eficiencia de la vigilancia apícola nacional. Además, en vista de la gran biodiversidad brasileña, es evidente que la ocurrencia y distribución de estos patógenos también debe investigarse en otras especies de abejas.
Palabras clave: Abejas; Apicultura; Microsporidia; Nosemosis; Salud apícola.

1. Introduction

Nosemosis is one of the main diseases that affect bees, being caused by microsporidia of the genus *Vairimorpha* (Microsporidia:Nosematidae), previously described as *Nosema* (Tokarev et al., 2020). Among the most prevalent etiological agents, and *Vairimorpha apis* and *V. ceranae* stand out, involving the Africanized bee *Apis mellifera* (Apidae:Apini) (Parrella et al., 2024). The pathogens have also been detected in other groups of bees, such as meliponas (Apidae:Meliponini), which are widely distributed in Brazil and the tropics (Porrini et al., 2017; Teixeira et al., 2020).

Vairimorpha spp. are obligate intracellular parasites that develop in the mucosal cells of the host's intestine, triggering degeneration of epithelial tissue and impaired cell renewal (Georgi et al., 2022). Among the main symptoms, digestive and nutritional symptoms stand out, such as diarrhea, changes in eating behavior (greater demand for food and amount ingested), weakness, changes in the composition of the microbiota, hormonal, physiological, and flight pattern changes, in addition to the premature death of bees (Zhang et al., 2021).

The pathogens are transmitted horizontally through the fecal-oral route and have a high virulence rate, being associated with great economic losses in infected apiaries (Parrella et al., 2024). Despite the high virulence rate, some studies reveal that the parasite can be present in bees for prolonged periods without causing symptoms in the individual or in the colony, remaining in a state of latency (Fernández et al., 2012; Lage et al., 2022). However, adverse conditions, such as coexistence with other pathogens (mites, bacteria, and viruses), deficiencies in colony management (lack of shading and nutritional deficiencies), and the presence of pesticide residues, can drive the development of nosemosis symptoms, even leading to the premature death of bees (Braglia et al., 2024; Castelli et al., 2020; Faita et al., 2021).

The disease has been reported worldwide, but its occurrence and distribution in Brazil are still poorly elucidated (Pires et al., 2016). Although the first record of *Vairimorpha* in the country was officially made in 2007 (Klee et al., 2007), the study by Teixeira et al. (2013) revealed that the pathogen has been present in Brazil for more than four decades, through molecular analysis of bee samples collected in 1979.

Some Brazilian apiaries have already been investigated for the presence of such pathogens, with reports in the central-west (Porrini et al., 2017), northeast (Lage et al., 2022; Nascimento et al., 2022; Neves et al., 2023), southeast (Guimarães-Cestaro et al., 2020; Santos et al., 2014; Teixeira et al., 2020) and south (Bizotto et al., 2018; Chagas et al., 2020; Nunes-Silva et al., 2016).

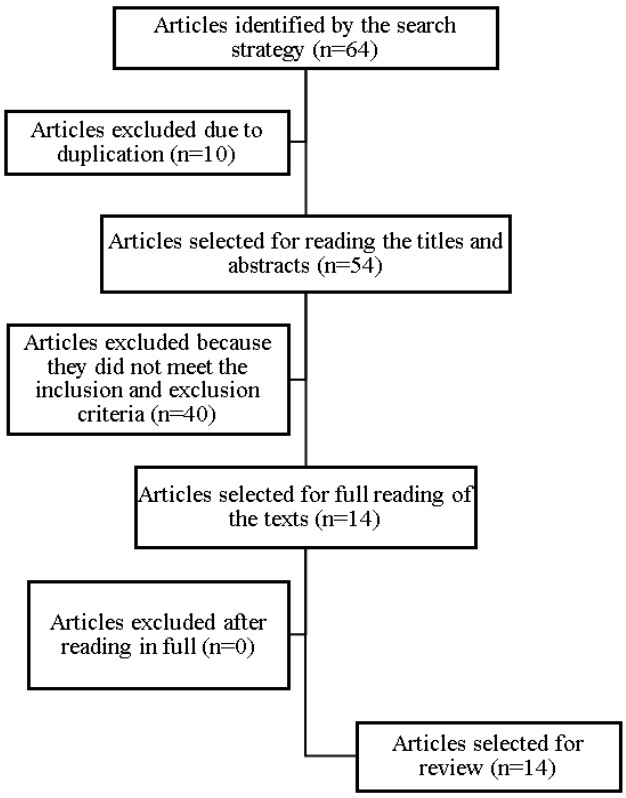
Despite the articles already published on the detection, and distribution of the pathogen *Vairimorpha* (*Nosema*) spp. in the country, no literature review brings together such studies and elucidates this theme. In addition, given the vast Brazilian territory, only a few states have had their apiaries investigated for the presence of such pathogens. It is therefore necessary to explain which Brazilian regions have this gap to be filled by the country's beekeeping surveillance and health systems. Thus, the present research is a systematic review of the literature on the occurrence and distribution of the microsporidia *Vairimorpha* (*Nosema*) spp. in apiaries in Brazil.

2. Methodology

The systematic review (Gomes & Caminha, 2014) was conducted based on the search for scientific articles on the occurrence and distribution of microsporum *Vairimorpha* (*Nosema*) spp. in apiaries in Brazil.

The study question was: what is the flow and distribution of the microsporidium *Vairimorpha* (*Nosema*) spp. in apiaries in Brazil? To this end, searches were conducted in the SciELO, PubMed, DOAJ and Capes Journals (Scopus) databases. The search strategy was the same in all databases. The keywords were searched in Portuguese, English and Spanish. The descriptors and terms consulted in the searches were: *Nosema*/ *Nosema*/ *Nosema* AND Brasil/ Brazil/ Brazil; *Vairimorpha*/ *Vairimorpha*/ *Vairimorpha* AND Brazil/ Brazil/ Brazil. No time limit was applied to the searches.

Figure 1 - Flowchart of searching and selecting articles process.



Source: Authors.

The selection of articles to compose this systematic review was carried out with the help of the online platform *Rayyan*. The following inclusion criteria were used: (I) Original scientific articles published in national and international journals; (II) Articles in Portuguese, English and Spanish; (III) Articles on the occurrence and distribution of the microsporidial *Vairimorpha* (*Nosema*) in Brazil; (IV) *In vivo* or *in vitro* experimental studies. The following studies were excluded: (I) In the form of letters, editorials, news, comments and case studies; (II) Articles not available in full; (III) Published in other languages; (IV) Review articles and book chapters. The following flowchart represents the process of searching and selecting articles (Figure 1).

3. Results and Discussion

After the search, 14 articles were selected, in Portuguese and English (Table 1).

Table 1 - Articles included in the review: bibliometric data, location of apiaries, bee species, pathogens evaluated, methodology and results.

References	Location of apiaries	Bee species	Pathogens evaluated	Methodology	Results
Bizotto et al. (2018)	Rio Grande do Sul	*Apis mellifera*	*Vairimorpha* sp.	Spore count via microscopy	Presence (+)
Chagas et al. (2020)	Rio Grande do Sul and Santa Catarina	*Apis mellifera*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (++)
Dias et al. (2024)	São Paulo and Minas Gerais	*Anthodioctes* spp. *Apis mellifera* *Centris analis* *Centris fuscata* *Centris tarsata* *Frieseomelitta varia* *Tetrapedia* spp.	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (+)
Guimarães-Cestaro et al. (2017)	São Paulo	*Apis mellifera*	*V. apis* *V. ceranae*	Spore counting via microscopy and PCR	Presence of *V. ceranae* (++)
Guimarães-Cestaro et al. (2020)	São Paulo	*Apis mellifera*	*V. apis* *V. ceranae*	Spore counting via microscopy and PCR	Presence of *V. ceranae* (++)
Lage et al. (2022)	Bahia	*Apis mellifera*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (++)
Nascimento et al. (2022)	Piauí	*Apis mellifera*	*Vairimorpha* sp.	Spore count via microscopy	Presence (+)
Neves et al. (2023)	Bahia	*Apis mellifera*	*V. apis* *V. ceranae*	Spore counting via microscopy and PCR	Presence of *V. ceranae* (++)
Nunes-Silva et al. (2016)	Rio Grande do Sul and Santa Catarina	*Apis mellifera;* *Melipona bicolor;* *Plebeia droryana;* *P. emerina;* *P. remota;* *P. saiqui; Tetragonisca fiebrigi*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (+) only in *A. mellifera*
Porrini et al. (2017)	Goiás	*Melipona fasciculata;* *M. quadrifasciata;* *M. marginata;* *M. rufiventris;* *M. mandaçaia;* *Tetragonisca angustula*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (++)
Santos et al. (2014)	São Paulo	*Apis mellifera*	*V. apis* *V. ceranae*	Spore counting via microscopy and PCR	Presence of *V. ceranae* (+++)

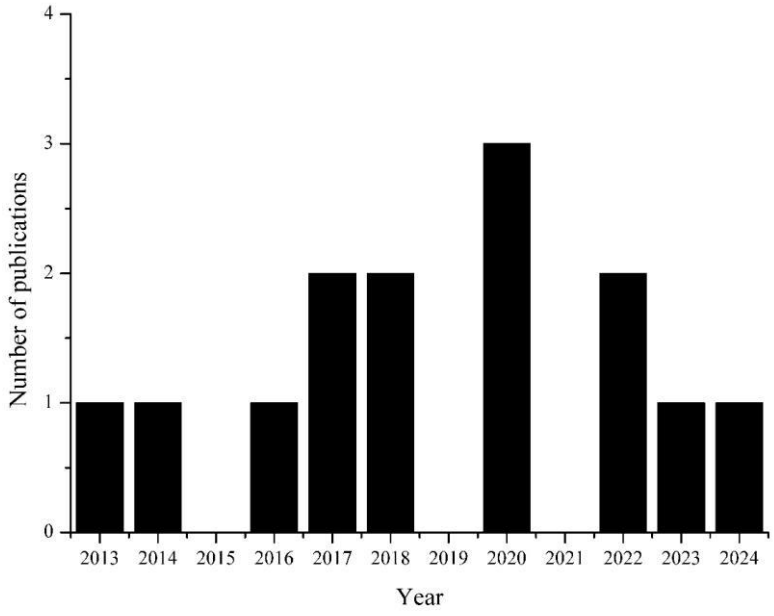
Teixeira et al. (2013)	Rio Grande do Sul	*Apis mellifera*	*V. apis* *V. ceranae*	Spore counting via microscopy and PCR	Presence of *V. ceranae* (++) and *V. apis* (++)
Teixeira et al. (2018)	São Paulo	*Apis mellifera*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (+++) and *V. apis* (+)
Teixeira et al. (2020)	Espírito Santo and São Paulo	*Friesella schrottkyi;* *Frieseomelitta varia;* *Tetragonisca angustula;* *Melipona marginata;* *M. quadrifasciata;* *M. mandaçaia;* *M. rufiventris;* *M. mondury;* *M. compressipes;* *Plebeia* spp.*;* *Scaptotrigona polysticta;* *S. xanthotricha;* *S. bipunctata*	*V. apis* *V. ceranae*	PCR	Presence of *V. ceranae* (+)

Pathogens in the samples evaluated: (+) little; (++) medium; (+++) high. Source: Authors.

This result aligns with the observed increase in biotechnological advancements during this period, and a deeper understanding of the CCD (Colony Collapse Disorder) phenomenon. Among the various factors contributing to the sudden decline of global bee populations, certain beekeeping diseases such as nosemosis, have emerged as significant causes (Nikita et al., 2022; Pires et al., 2016).

The studies were published from 2013 to 2024, and many publications occurred from 2017 onwards (Figure 2).

Figure 2 - Number of publications found in the search, classified by year.



Source: Authors.

The studies identified in the search were conducted in apiaries across eight Brazilian states: Bahia, Espírito Santo, Goiás, Minas Gerais, Piauí, Rio Grande do Sul, Santa Catarina, and São Paulo, and four regions: Midwest, Northeast, Southeast, and South (Table 1 and Figure 3). It is noted that the majority of research is concentrated in the Southeast and South

regions, with a particular emphasis on São Paulo (six publications) and Rio Grande do Sul (four publications) (Table 1 and Figure 3).

Given Brazil's vast territory, many areas have yet to have their apiaries investigated for the presence of the pathogen *Vairimorpha* spp., particularly in the northern region of the country (Table 1 and Figure 3). According to IBGE (2022), Brazil produced 60,966,305 kg of honey in 2022, equivalent to R$ 957,811.00, with the largest producers being the states of Rio Grande do Sul, Paraná, São Paulo, Minas Gerais, and Piauí, corresponding to the south, southeast and northeast regions. In contrast, the region with the lowest production was the North of the country, where the lowest values were concentrated in the States of Amazonas, Amapá, and Acre. Correlating the IBGE data (2022) with the present study, we can observe that the regions with a higher honey production consequently play a greater socioeconomic influence and, thus, concentrate a greater number of research linked to these production chains. Also in this context, this review highlights the places that deserve greater attention from the country's beekeeping surveillance and health systems, considering the major socioeconomic impacts that can be associated with nosemosis.

Figure 3 - Brazilian states with publications found in the search, highlighted in gray.

Source: Authors.

For bees, ten of the selected studies evaluated exclusively the species *A. mellifera* and the other four were native Brazilian bee species (Table 1). A total of 26 species were investigated (Table 1). Considering the wide Brazilian biodiversity, since the number of bee species described in Brazil exceeds 3,000 (Silveira et al., 2002) and, worldwide, about 20,000 (Spiesman et al., 2024), in this review the articles only explored less than 1% of this total. In this scenario, further studies are needed to evaluate the health of the apiaries of Brazilian native bees, to elucidate the subject and control the quality and health of these apiaries.

The fact that the Africanized bee *A. mellifera* was the most investigated in the selected studies is compatible with its great socioeconomic importance in Brazil and in the world (Frazier et al., 2024). In addition to the significant influence of its

pollination on agricultural production (Khalifa et al., 2021), *A. mellifera* is also the main producing species of honey, propolis, wax and derivatives (Baena-Díaz et al., 2022). In Brazil, it was the first bee species investigated for infection by the microsporidia *Vairimorpha* spp. (Klee et al., 2007).

Regarding the pathogens evaluated, only the species *V. apis* and *V. ceranae* were investigated in the selected publications (Table 1). This result is consistent with the fact that these are the main species that infect bees, especially the Africanized bee *A. mellifera* (Parrella et al., 2024). However, other relevant species also infect bees, such as *Vairimorpha bombi*, identified mainly in the genus *Bombus* (Webster et al., 2024), which reveals a gap to be investigated in future research.

The selected articles employed the next methodologies: molecular analysis using Polymerase Chain Reaction (PCR) and microbiological analysis through spore counting via light microscopy (Table 1). In the studies, it can be observed that the techniques were used together (five articles) or individually (seven for PCR and two for microscopy) (Table 1). The PCR technique has several variations (examples: multiplex, real-time, and digital) and was the most used in the articles, which is consistent with the fact that it is considered the gold standard for detecting the pathogen *Vairimorpha*, due to its greater sensitivity and precision (Truong et al., 2021). Other methodologies that were not addressed can also be used, such as scanning electron microscopy (SEM), loop-mediated exothermic amplification (LAMP), chromatography (gas and liquid), and immunofluorescence (Fries et al., 2013; Mazur & Gajda, 2022).

Among the results obtained in the articles, it can be observed that most of the studies detected the presence of *V. ceranae* in the analyzed samples and only two found the pathogen *V. apis* (Table 1). In addition, in positive detection cases, the intensity was higher for *V. ceranae* (Table 1). This result is consistent with the literature, which reveals that the microsporidium *V. ceranae* is widespread in South America (Martínez et al., 2012; Pacini et al., 2021), including Brazil (Lage et al., 2022; Santos et al., 2014), being much more prevalent and detected than *V. apis* in these regions.

The phenomenon "*Colony Collapse Disorder*" (CCD) has been reported worldwide, with major environmental and socioeconomic impacts associated with bee decline (Nikita et al., 2022). In Brazil, significant losses of bee populations have already been reported and, among the possible causes, pests and diseases, such as nosemosis, stand out (Freitas et al., 2023; Pires et al., 2016). However, few studies are available in this regard, without official data. In this scenario, it is increasingly necessary to do further studies to elucidate the subject, investigating the possible causes of CCD in Brazil and the frequency in which this phenomenon occurs.

Biotic and abiotic factors such as climate change derived from global warming (Manlik et al., 2022), the unavailability of food and natural resources (Castelli et al., 2020), the emergence of other pathogens and parasites (viruses, bacteria, and mites) (Chagas et al., 2020), and the abusive use of pesticides (Faita et al., 2020), are considered determinant stressors for the development of nosemosis (Sabolová et al., 2024), which could lead to an epidemic of this disease in the country. Understanding the distribution of the pathogen in Brazil enables the development of targeted management strategies within national beekeeping health programs. This understanding is crucial for formulating preventive public policies that incorporate periodic disease monitoring.

4. Final Considerations

Based on the articles selected in this review, only eight Brazilian states had their apiaries investigated for the presence of the microsporidial *Vairimorpha* spp. (Bahia, Espírito Santo, Goiás, Minas Gerais, Piauí, Rio Grande do Sul, Santa Catarina and São Paulo). No studies were conducted in the northern of the country, with most of the research concentrated in the southern and southeastern regions. Regarding the *Vairimorpha* species, only the species *V. apis* and *V. ceranae* were

investigated, and *V. ceranae* was predominantly detected. Other relevant species, such as *V. bombi*, were not evaluated. Most studies focused exclusively on the bee species *A. mellifera*, except for four articles that examined other bee species.

This review highlighted Brazilian regions that have yet to be explored for the incidence and distribution of these pathogens, revealing a gap that needs to be addressed by the country's beekeeping surveillance and health systems. Furthermore, considering Brazil's rich bee biodiversity and the fact that only about 1% of the species have been studied, it is evident that many species remain unexamined.

Conflict of Interest

The authors declare no competing interest.

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