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First Detection and Molecular Characterization of *Nosema ceranae* Isolated from Honey Bees (*Apis mellifera*) from Apiaries in the Northern Highlands of Ecuador

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Simple Summary

Bees play a key role in agriculture and the environment since they pollinate many plants that provide food for people and animals. However, their health can be affected by microscopic parasites that cause diseases and weaken colonies. In Ecuador, little was known about which of these parasites were affecting honeybees. This study investigated the presence of two species of *Nosema*, a group of tiny organisms that infect bees and can reduce honey production and colony survival. Samples were collected from different provinces in the northern region of the country, and laboratory tests showed that both species, *Nosema apis* and *Nosema ceranae*, are present in Ecuador. The second species was found more frequently and is closely related to those found in other South American countries. This is the first report confirming the presence of both *Nosema* species in Ecuador. The findings are for food security and environmental sustainability.

Abstract

The development of beekeeping in Ecuador has generated the need to strengthen the bee health program. Research on the main pathogens responsible for diseases like nosemosis, which can severely impact bee health, is of special interest. This study aims to detect and identify the *Nosema apis* and / or *Nosema ceranae* species affecting honeybee colonies located in the northern Sierra region of Ecuador through the amplification of RPB1 gene fragments by multiplex PCR and the phylogenetic analysis of *N. ceranae* based on the 16 S RNAr gene. Of the 164 honeybee samples collected from colonies located in the provinces of Carchi, Imbabura and Pichincha, *N. apis* was detected in 14.63%

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(24/164), and *N. ceranae* in 21.34% (35/164). Phylogenetic analysis showed that *N. ceranae* from Ecuador is closely related to the sequences from Argentina and Brazil. This study is the first in the country to report the presence of these two microsporidia species, being valuable for improving diagnostic capabilities.

Keywords: Nosemosis; *Nosema*; *N. ceranae*; *N. apis*; *RPB1*gen and *16S RNAr* gen

1. Introduction

Beekeeping activity in Ecuador has been growing steadily. In 2016, a total of 902 apiaries and 12188 colonies of domestic were registered, with most of them concentrated in the Sierra region (mountain area); where the provinces of Pichincha (22.79%), Imbabura (8.41%) and Carchi (7.99%) have the highest numbers of colonies [1]. Given the increase in this activity in the country, the Agencia de Regulación y Control Fito y Zoosanitario de Ecuador (AGROCALIDAD) aimed to get information regarding the health status of those colonies. They conducted a nationwide study of the main pathogens affecting honey bee colonies and reported the presence of *Nosema* sp. in 235 of the sampled apiaries. However, the species was not identified [1].

Microsporidia of the genus *Nosema* are intracellular parasites [2] with more than 150 described species [3] affecting both mammals [4] and insects [5] especially of the order Hymenoptera and Lepidoptera [6]. Nosemosis is a disease caused by the microsporidia *Nosema apis* [7] and/or *Nosema ceranae* [8–10] which has a worldwide distribution [11–15]. *Nosema apis* and *Nosema ceranae* have recently been reclassified as *Vairomorpha* [16] although its reclassification is still under discussion [17]. For this reason, several authors still retain the previous nomenclature of *Nosema* [18–20], while others have already adopted the new name *Vairomorpha* [21–24]. In this study, we will use the both name *Nosema* and *Vairomorpha*.

Within the Apidae family, the first report of infection was reported in 1996 by *Nosema ceranae* in *Apis cerana* [10]. As for *Apis mellifera*, the first report of *N. ceranae* as a new pathogen was reported in 2006 [8,25].

In the South American region, several countries such as Brazil [26], Argentina [27], Chile [28] or Uruguay [29] have reported the presence of these parasites in their colonies [30]. Within the Caribbean, Dominica Island reports the presence of both species [31], and to the north of the American continent the presence of this microsporidium was reported in Mexico [32], USA and Canada [33]. In addition, *N. ceranae* has been found in bumblebees of the genus *Bombus* [34,35] and in native stingless bees [36].

N. apis and *N. ceranae* can enter the honey bees by ingestion of spores with the food or by cleaning the alveoli and the inside of the hives [37–40]. The spores invade the cells of the intestinal wall of bees and multiply [41–44]. Traces of spores have also been found in other parts of the body, such as hypopharyngeal gland tissue, salivary glands, Malpighian tubules and fat body [45]. Infection by either or both *Nosema* species, can cause alterations in behavior [46], metabolism and nutrition [47], decreased bee life expectancy and mortality [48], as well as decreased honey production [49].

Reported prevalence levels of nosemosis varied depending on the country, the season and the species of the causal agent. For example, in Canada, prevalence of *N. apis* can range between 4-34% [50], in México 80-90% while *N. ceranae* was about 12-13% in Mexico [51], 58% in Saudi Arabia [52] or up to 66% in Romania [53]. Co-infections have also been recorded in hives from Turkey, the study carried out since 2009 to 2016, in different seasons, reports that the prevalence varies from 20-49% in samples [54].

Among the methods developed for *Nosema* diagnosis described in the OIE Terrestrial Manual [39] and in Standard methods for *Nosema* research [55] are microscopic observation of spores, fluorescence microscopy and Polymerase Chain Reaction (PCR), the latter being the most employed because under the light microscope it is difficult to differentiate morphologically these two *Nosema* species. Primers based on the 16S rRNA gene described by [56] are widely used [14,57,58]. However,

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authors such as Gisder and Genersch [59] have chosen to develop or use primers based on the large subunit of the RNA polymerase II gene (RPB1) to differentiate *Nosema* species [59–61], which is also used to observe population diversity [62] and genetic diversity [63] in *N. apis* and *N. ceranae*.

The aim of this study was to detect and differentiate the type of microsporidium that causes nosemosis in domestic colonies of honey bees in the northern highlands of Ecuador, using a multiplex PCR based on the RPB1 gene. In addition, we present the first molecular characterization of *Nosema ceranae* in apiaries in Ecuador based on the 16S rRNA gene.

2. Materials and Methods

2.1. Sample Collection

Based on the data obtained from the first beekeeping census carried out by AGROCALIDAD (2016) [1], the study area focused on the provinces of Pichincha and Imbabura given the greater concentration of apiaries (a) and hives (h) in the northern part of the Ecuadorian Sierra, and Carchi because it is the border province with Colombia.

Between the months of April to June 2017, samples of foragers honey bees were collected from the hive entrance (h=164), located in apiaries (a=29) in the three intervened provinces (Table 1). The location of the apiaries was recorded with the help of a Garmin 60Cs GPS and the EpiCollect 5 application.

Table 1. Distribution of existing and sampled apiaries and beehives in the three provinces surveyed.

Province	Number and percentage of apiaries of the national total[a]	Number and percentage of apiaries Sampling	Number and percentage of beehives of the national total[b]	Number and percentage of beehives Sampling
Carchi	40 (4,43%)	4 (10%)	974 (7,99%)	33 (3,39%)
Imbabura	74 (8,20%)	13 (17,58%)	1025 (8,41%)	68 (6,64%)
Pichincha	108 (11,97%)	13 (12,04)	2778 (22,79)	63 (2,28%)
Total	222/902[a] (24,61%)	30 (13,51%)	4777/12188 [b]	164 (3,43%)

[a] Apiaries at national level; [b] Number of hives nationwide.

2.2. Diagnostic Tests

For the diagnosis of *Nosema* sp. in honey bees, light microscopy and PCR laboratory tests were used. Each of the 164 samples was individually analyzed with both techniques. In addition, the florescence microscopy test was used on one of the samples diagnosed as co-infected by PCR to observe and compare the size of *N. apis* and *N. ceranae* spores.

2.3. Optical Microcopy Test and Determination of Spore Number

Abdomen from approximately 20 honey bees per colony were aseptically separated with forceps and scalpel, mixed with 1 mL of distilled water, macerated and placed in vials. An aliquot of the sample (10 µL) was placed on a Neubauer chamber and visualized with an optical microscope at 400X magnification.

The spore concentration was obtained by multiplying the average number of spores in the sample by the dilution factor and dividing by the product of the chamber area (mm) by the chamber depth (mm). The level of bee infestation was then classified according to the following scale: low

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(<1,000,000 spores/bee), medium (>1,000,000 <2,000,000 spores/bee) and high (more than 2,000,000) [64].

2.4. DNA Extraction of Nosema sp. in Honey Bees

The protocol used for DNA extraction was the described by Hamiduzzaman et al., (2010) [65] with modifications. The abdomens of 20 honey bees from each colony were placed in 2 mL vials. 500 µL of extraction buffer (0.03 M CTAB, 0.05 M Tris, 0.01 M EDTA, 1.1 M NaCl, pH 8.0), and 4 µL of Proteinase K (20 mg/ml) were added. Samples were triturated with a sterile pistil, vortexed, and incubated at 60 °C for 3 h with constant shaking, occasionally inverting the tubes during incubation. They were then centrifuged for 1 min at 14000 rpm, and the supernatant was transferred to a 1.5 mL vial. A double extraction with phenol-chloroform (1:1) was performed by adding 300 µL of this mixture, homogenizing the tubes by inversion and centrifuging them at 14000 rpm for 15 min; the supernatant was transferred to a new vial. Then 300 µL of chloroform was added and centrifuged at 10000 rpm for 5 min. 30 µL of 3 M sodium acetate and 600 µL of 95 % ethanol were added to the supernatant, mixed gently and stored at -20 °C overnight. The samples were centrifuged at 10000 rpm for 10 min and the ethanol was discarded. Subsequently, 1 mL of 75% ethanol (4 °C) was added and mixed briefly by vortexing. The pellet was then centrifuged for 3 min at 14000 rpm, the ethanol was discarded, and the pellet was allowed to dry. Finally, the DNA pellet was re-suspended in 100 µL of ultrapure distilled water free of nucleases, and the samples were incubated in a water bath at 65 °C for 10 min. Samples were incubated with RNAse at 37 °C for 10 min. The extracted DNA was stored at -20 °C until use.

2.5. Detection and Identification of Nosema sp. by Multiplex PCR

Obtained DNA was subjected to multiplex PCR, using two pairs of primers (Table 2), that amplify different regions of the RPB1 gene, depending on the *Nosema* species. The multiplex PCR assay consisted of adding 1X of PCR buffer, 0.5 µM of primers NosaRNAPol-F2 / NosaRNAPol-R2, for the detection of *N. apis*; 0.4 µM of primers NoscRNAPol-F2 / NoscRNAPol-R2 for *N. ceranae*; 1.75 mM MgCl2, 0.8 mM dNTP mix (0.2 mM/dNTP), 1.25 U/µL Taq polymerase enzyme (Invitrogen), 400 ng DNA, plus a volume of UltraPure ™ DNase/RNase-Free Distilled Water to complete 25 µL of reaction. Cycling conditions in the thermal cycler were 95 °C initial denaturation for 5 minutes; 40 one-minute cycles of denaturation steps at 94 °C, annealing primer 67 °C and extension at 72 °C, and a final extension cycle at 72 °C for 10 minutes. Additionally, a single PCR assay (Table 2) was performed on *N. ceranae* positive samples according to the results of the multiplex PCR. A 218 bp fragment of the 16S rRNA gene was amplified, following the protocol described by Higes et al. (2006) [8].

Table 2. Primers used for the detection and phylogenetic analysis of *N. apis* and *N. ceranae*.

Primer name	Sequence (5′-3′)	Species	Fragment size
NosaRNAPol-F2*	AGCAAGAGACGTTTCTGGTACCTCA	*Nosema apis*	297 bp
NosaRNAPol-R2	CCTTCACGACCACCCATGGCA		
NoscRNAPol-F2*	TGGGTTCCCTAAACCTGGTGGTTT	*Nosema ceranae*	662 bp
NoscRNAPol-R2	TCACATGACCTGGTGCTCCTTCT		
218MITOC-FOR**	CGGCGACGATGTGATATGAAAATATTAA	*Nosema ceranae*	218-219 bp
218MITOC-REV	CCCGGTCATTCTCAAACAAAAAACCG		

bp: base pairs; *Primers for amplification of RPB1 gene fragments [59]; ** Primers for 16S rRNA gene fragment amplification [8].

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2.6. Sequencing, Molecular Characterization and Phylogenetic Analysis

After molecular detection of *Nosema* species by multiplex PCR, phylogenetic analysis of *N. ceranae*, was performed, as the species of greatest interest, based on the 16S rRNA gene primers (Tabla 2). Only those *N. ceranae* positive samples with a strong band intensity were chosen, and the products of the single PCR assay were sent for sequencing, in duplicate and in both directions by the Sanger method to Macrogen® Korea. Consensus sequences (n = 9) from Ecuador were compared with sequences of isolates available in GenBank.

A phylogenetic tree was constructed to determine the phylogenetic relationship between *N. ceranae* isolates from Ecuador and sequences belonging to Americas, Europe and Asia, as well as to observe the relationship between the sequences from this study and other sequences within the *Nosema* genus. The tree was constructed in MEGA 12 program using the consensus sequence from this study and the sequences available in GenBank. The maximum parsimony method, which uses the subtree-pruning-regrafting (SPR) algorithm), was employed for the analysis. This analysis involved 24 nucleotide sequences. There was a total of 222 positions in the final dataset. *Trachipleistophora hominis* was used as the outgroup.

2.7. Detection of Spores by Fluorescence Microscopy

Sample A27C2 was subjected to complementary analysis by fluorescence microscopy according to the modified procedure of Snow(2016) [66]. Smear samples of bee macerates were incubated for 2 h at room temperature in 3% glutaraldehyde (60 µL). To remove the fixative, two washes were performed, for 10 min, with 1 mL of PBS-T solution (PBS + 0.01 %Triton X-100). 500 µL of fluorescent FB28 (Calcofluor White M2R) was added. After overnight incubation at 4 °C in a humid chamber, the plates were washed twice with PBS-T solution. Next, as a co-stain, 200 µL of Hoesch DNA dye (1:2000) was added and incubated for 5 min at 4 °C in humid chamber. The plates were washed twice with PBS-T solution. Then, the plates were dried at room temperature in the dark, observation was performed on an Olympus IX53 microscope at 384.6 ms exposure in 20X lens.

3. Results

3.1. Detection of Nosema sp. Spores by Optical Microscopy

The oval shape of *Nosema* sp. spores was generally observed by light microscopy without identification of the species (Figure 1). The prevalence of *Nosema* sp. was 41.38 % (12/29) at the apiary level and 17.07 % (28/164) at the colony level.

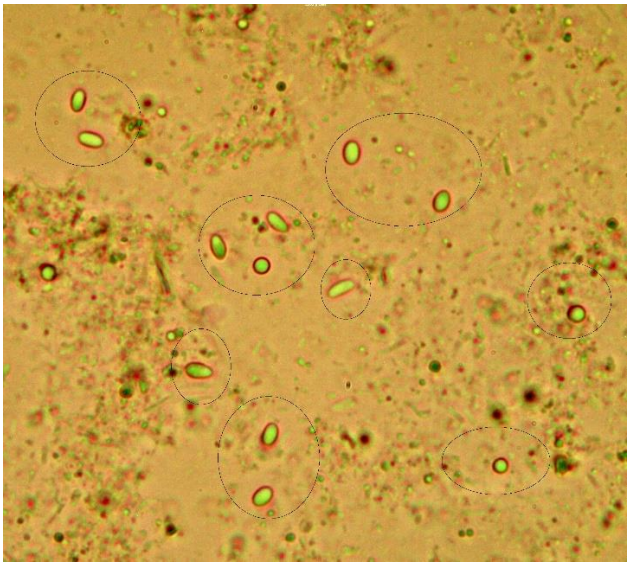

Figure 1. Spores (black circles) of *Nosema* sp. by optical microscopic examination (400X).

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The province of Pichincha (h = 63) had the highest number of positive samples (20/63), followed by the province of Carchi (5/33) and finally Imbabura (3/68) (Table 3). On the other hand, low (h = 9), medium (h = 2) and high (h = 17) levels of infestation or spore intensity were observed.

3.2. Identification of Nosema apis and Nosema ceranae by PCR

By multiplex PCR we detected *Nosema* sp. infection in 34.76% (59/164) of colonies and 86.21% (25/29) of apiaries. We determined the presence of *N. apis* and *N. ceranae* in the colonies of the three provinces with a prevalence of 14.63% (24/164) and 21.34% (35/164) respectively, finding also apiaries (a = 5) and colonies (h = 2) with double infections.

Among the three provinces, Pichincha had the highest prevalence of both *N. apis* (24.24 %) and *N. ceranae* (36.50 %) at the colony level. While in Imbabura and Carchi there was a higher prevalence of *N. apis* (14.70 % and 12.69 % respectively) than *N. ceranae* (13.23 % and 9.09 % respectively) in the colonies (Table 3). Table 3 gives details of the distribution of results (number, prevalence, and 95 % confidence intervals) for light microscopy and PCR tests, at the apiary, colony and provincial level.

3.3. Molecular Characterization and Phylogenetic Analysis of N. ceranae

BLAST analysis of the fragment sequence was 99.5-100 % identity with partial sequences of small subunit ribosomal RNA gene isolates. Nine sequences of *N. ceranae* (n = 9) were obtained from various sectors of the provinces of Imbabura (n = 3) and Pichincha (n = 6), accession numbers: PQ336918, PQ336919, PQ336920, PQ336921, PQ336922, PQ336923, PQ336924, PQ336925, PQ336926. Since all of them showed 100 % homology, only one sequence was used in the phylogenetic tree (PQ336918).

The phylogenetic analysis involved nine species of microsporidia belonging to this genus (Figure 2). We observed that the isolates from Ecuador are located within the same clade of *N. ceranae*, confirming that they belong to this species. Furthermore, the closest species are *N. bombi* and other *Nosema* species, leaving *N. apis* distantly related to *N. ceranae*.

Within of the *N. ceranae* clade (Figure 2), we don't observe a grouped regionalization by continent. Thus, sequences from American continent are distributed among all *N. ceranae* subclades. Consequently, the isolate from Ecuador is placed at the same phylogenetic distance as isolates from Argentina and Brazil (South America), Saudi Arabia and Iran (Asia), and Spain and Lithuania (Europe).

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Table 3. Frequency of *Nosema* sp. by microscopy and multiplex PCR in the analysis of apiaries and hives and by each province.

Province	Number apiaries	Microscopy		PCR		Total hives	Microscopy		PCR	
		Number – prevalence in % (95% CI) *Nosema* sp.	Number - prevalence in % (95% CI) *N. apis*	Number – prevalence in % (95% CI) *N. ceranae*	Number - prevalence in% (95% CI) Co-infection		Number – prevalence in % (95% CI) *Nosema* sp.	Number – prevalence in % (95% CI) *N. apis*	Number – prevalence in % (95% CI) *N. ceranae*	Number – prevalence in % (95% CI) Co-infection
Carchi	4	2 - 50% (6.76 – 93.24)	3 - 75% (19.41 – 99.37)	1 - 25% (0.63 – 80.59)	1 - 25% (0.63 – 80.59)	33	6 - 18.18% (6.98 – 35.46)	6 – 18.18% (6.98 – 35.46)	3 - 9.09% (1.92 – 24.33)	1 - 3.03% (0.08 – 15.76)
Imbabura	13	3 - 23.08% (5.04 -53.81)	5 – 38.46% (13.86 – 68.42)	7 – .15% (19.22 -74.87)	0 -*	68	3 - 4.41% (0.92 – 12.36)	10 – 14.71% (7.282- 25.39)	9 - 13.24% (6.33 – 23.64)	0 -*
Pichincha	12	7 - 58.33% (27.67 - 84.83)	4 - 33.33% (9.92 – 65.11)	8 - 66.67% (34.89 – 90.08)	2 – 8.33% (0.21 – 38.48)	63	19 - 30.16% (19.23 – 53.02)	8 - 12.70% (5.65 - 23.50)	23 - 36.51% (24.73 – 49.6)	1 - 1.59% (0.04 – 8.53)
TOTAL	29	12 – 41.38% (23.52 – 61.06)	12 – 41.38% (25.52 – 61.06)	15 – 51.72% (32.53 – 70.55)	2 – 6.90% (0.85 – 22.77)	164	28 - 17.07% (11.65 – 23.72)	24 - 14.63% (9.61 – 20.99)	35 - 21.34% (15.34 – 28.41)	2 - 1.22% (0.15 4.34)

*: IC does not apply.

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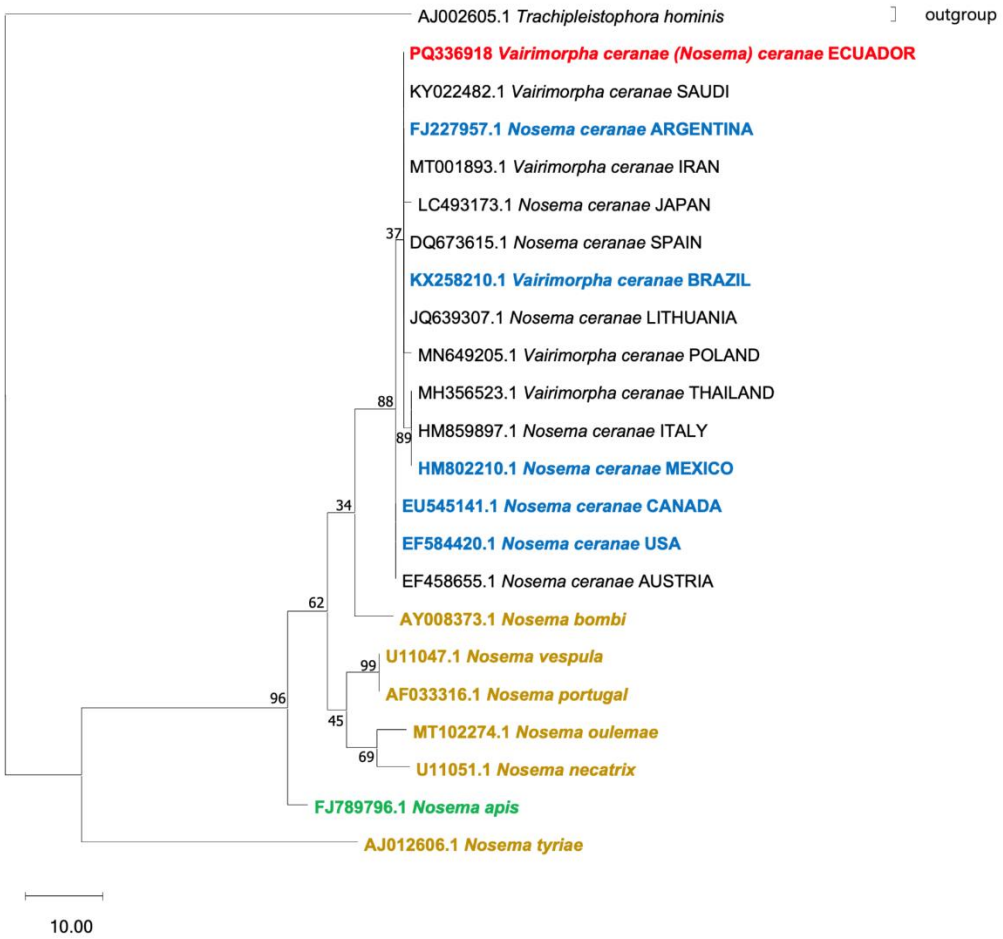

Figure 2. Phylogenetic tree of microsporidia *Nosema* based on the sequences of the small subunit rRNA and constructed by Maximum Parsimony analysis. Blue: *N. ceranae* from Americas; gold: other *Nosema* species; green: *N. apis*; black: *N. ceranae* from continents other than Americas; red: *N. ceranae* from Ecuador. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The consistency index is (0.838), the retention index is (0.883), and the composite index is 0.783 (0.740) for all sites and parsimony-informative sites (in parentheses).

3.4. Detection of Nosema sp. by Fluorescence Microscopy

Through fluorescence microscopy (Figure 3), the oval forms of *Nosema* sp. spores were visualized, being between 4 µm and 6 µm long, stained with the Hoesch dye which can stain the DNA of the cells and additionally it was possible to take the approximate measurements of them. The red staining of the spores indicates a positive result to the FB28 dye or calcofluor which is specific for chitin, polysaccharide component of the fungal cell wall. This specific staining allows us to confirm definitively that they are *Nosema* sp. microsporidia.

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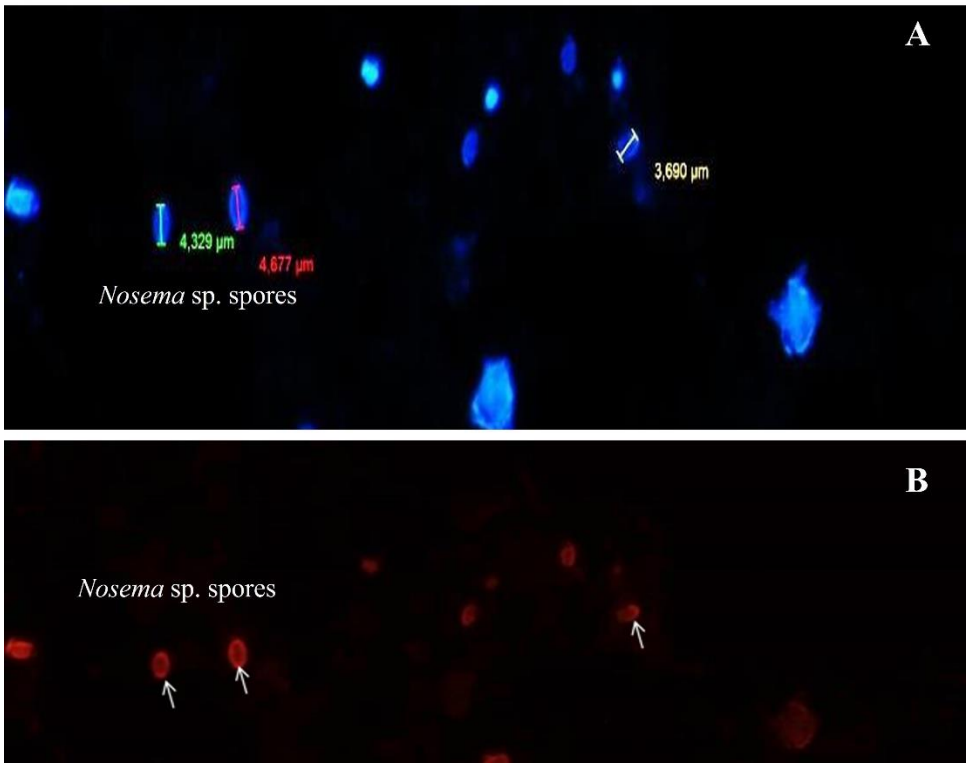

Figure 3. Spores of *Nosema* sp. observed by Olympus IX53 fluorescence microscopy. *Nosema*'s spores stained with A) Hoesch dye and B) FB28 red dye at 40X.

4. Discussion

This study revealed the presence of *N. apis* (14.63 %) and *N. ceranae* (21.34 %) in colonies from Ecuadorian territory. The prevalence of both microsporidia species results higher than reported by the regulatory agency (9 % / 235 apiaries sampled) in the 24 provinces of Ecuador [1]. Visualization of spores by light microscopy technique is relatively straightforward, experts may be able to discern between spores [67], with fluorescence microscopy it has been shown that calcofluor, specifically binds to chitin in the cell wall of mature spores [66]. Immature spores lack chitin, this method is useful for studies of mature spore localization within cells, but cannot differentiate between species [66].

Although the light microscopy technique is used as an initial screening for spore detection, diagnosis and differentiation of *Nosema* species has been carried out based on molecular methods such as PCR and its variants [68,69], because of its sensitivity and specificity [55,70], which is clearly observed in this research. Thus, it is proposed that this type of technique should be incorporated into regulatory agencies for greater accuracy in distribution and prevalence studies.

Although the presence of *Nosema* spp. has been previously reported in Ecuador [71,72], as a thesis of universities in the country, these studies were carried out using light microscopy, so the species of *Nosema* are not differentiated. Therefore, the present study is the first report of the distribution of *N. apis* and *N. ceranae* in the northern part of Ecuador with molecular characterization. Hence, both pathogens are present in the provinces studied. Furthermore, the prevalence of *N. ceranae* determined here is in addition to reports where higher prevalence than *N. apis* are expressed [45,49,51,73,74].

The detection of *Nosema* spp. in bees using calcofluor white and Hoechst staining proved highly effective, allowing for the identification of fluorescent spores measuring 4-6 µm. Calcofluor specifically stained the chitin in the spore wall, while Hoechst confirmed the presence of DNA in the nucleus, facilitating spore differentiation. These fluorescent stains, sensitive and rapid, outperform traditional methods such as phase-contrast microscopy, making them valuable for diagnosis and epidemiological monitoring [66,75].

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The maximum parsimony using the 16S rRNA gene determined that there is no difference between geographically distant isolates within the South American continent. However, they are also related to sequences from Iran (Asia) and Lithuania (Europe). The finding agrees with those of [76] where they found identical haplotypes from samples originating from Spain, Slovenia and Kyrgyzstan. Molecular phylogeny using the small subunit revealed that *N. apis* and *N. ceranae*, pathogens of the same host, are highly divergent, and are not very close relatives within the genus *Nosema* [38,45,62].

The fact that *N. apis* and *N. ceranae* microsporidia are also present in hives in Ecuador contributes to a better understanding of the dynamics of diseases affecting the bee population. This will allow the implementation of specific preventive measures, adapted to the country's context, to control their spread.

5. Conclusions

This is the first report of *N. ceranae* in Ecuador. The prevalence found and the co-infections with *N. apis*, suggest a wide census *of Nosema* species throughout Ecuador. The detection of the two *Nosema* species in the apiaries and in the colonies shows the importance of contagion within the apiaries. Future studies using haplotype networks could determine if there are genetic variants infections between apiaries.

This study represents the first report of *Nosema ceranae* in Ecuador, highlighting its presence alongside *Nosema apis* and suggesting a broad distribution of *Nosema* species across the country. The observed co-infections underscore the potential for significant pathogen exchange within and between apiaries, emphasizing the need for improved biosecurity measures. The detection of both *Nosema* species within colonies also raises concerns about the potential cumulative effects on colony health and productivity.

Future research should prioritize molecular studies, such as haplotype network analyses, to explore the genetic diversity of *Nosema* populations and identify transmission pathways. Additionally, longitudinal studies on colony dynamics could clarify the impact of co-infections on bee health and survival. Implementing large-scale monitoring programs in Ecuador and globally will be essential to assess the prevalence and spread of *Nosema* species, enabling the development of targeted control strategies. These efforts could significantly contribute to safeguarding apiculture and ensuring pollination sustainability worldwide.

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Abbreviations

GISAH	Grupo de Investigación en Sanidad Animal y Humana
RPB1	RNA Polymerase II Subunit RPB1
16 S RNAr	16S ribosomal RNA
PCR	Polymerase Chain Reaction
N. apis	*Nosema apis*
AGROCALIDAD	Agencia de Regulación y Control Fito y Zoosanitario (Ecuador)
N. ceranae	*Nosema ceranae*

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OIE	*World Organization for Animal Health*
GPS	Global Positioning System
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
RNAse	Ribonuclease
dNTP	Deoxyribonucleoside Triphosphate
RNA	Ribonucleic Acid
USA	United States of America

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