

# Nosema distribution across Portugal.

## Results from the first nationwide survey (2011-12)

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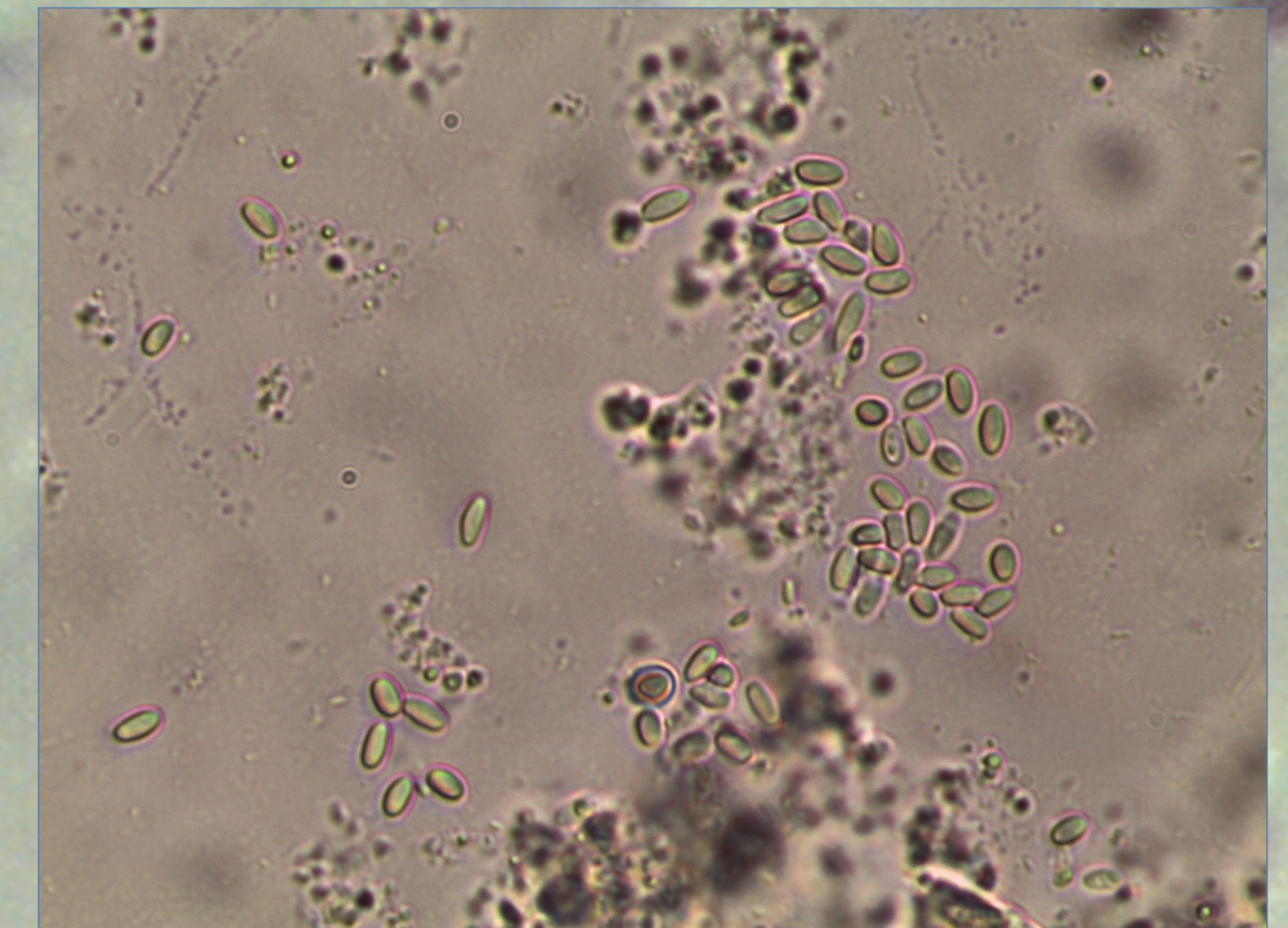
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## Introduction

Nosemosis is not a recent pathology in Portugal. The etiological agent traditionally associated with it, the microsporidian *Nosema apis*, is well characterized and was not causing considerable economic damage to the Portuguese apiculture. However, the recent arrival, to the 'European' honeybee sphere, of a 'new' *Nosema* species (*N. ceranae*) has been recurrently considered a relevant contribution to the 'Colony Collapse Disorder' (CCD) and its potential to be a 'game changer' across the Iberian peninsula has justified the present study in Portugal.

Therefore, a nationwide field sampling exercise was launched in 2011, to address mounting anecdotal evidence of atypically high honey bee colony mortalities of unexplained origin occurring throughout Portugal. The first approach was to contact 662 beekeepers ( $\approx 4\%$  of the registered Portuguese beekeepers), via telephone interviews, with a view to formulating an 'educated guess' regarding the cases where *N. apis* / *N. ceranae* seemed more likely to have had a role in colony mortality/morbidity. This study appears in the context of a project submitted by FNAP Measure 6A National Beekeeping Program (under EU Regulations No. 917/2004, No. 797/2004 and No. 1234/2007), in partnership with research institutions of the authors, under coordination of Prof. Sância Pires.



## Methodology

Following these interviews, a total of 277 apiaries ( $\approx 3$  sampling sites per county), representing the whole continental part of the country, were sampled according to standard methodology (pooled samples of 60 foragers from the outside the hive entrance). The abdomen of these individuals are macerated in 10 ml of water using a mortar and pestle. All samples were assessed both by standard light microscopy (in a first approach to *Nosema* spore presence/absence) and by molecular PCR-based methodology (mainly aiming at *Nosema* spp. identification), basically following de OIE (2008) recommendations.

Primers for the *Nosema* 16S rRNA gene were MITOC-F (5' CGGCGACGATGTGATATGAAAATATTA 3') and MITOC-R (5' CCCGGTCATTCTCAAACAAAAACCG 3') that yield a 218 bp PCR product specific for *N. ceranae* and APIS-F (5' GGGGGCATGTCTTTGACGTA 3') and APIS-R (5' GGGGGCGTTTAAATGTGAAACAATATG 3') that yield a 321 bp PCR product specific for *N. apis*.

Additionally, the primer pair RpS5-F (5' AATTATTTGGTCGCTGGAATTG 3') and RpS5-R (5' TAACGTCCAGCAGAATGTGGTA 3') (115 bp length product) were used in all reactions as a standard control (Hamiduzzaman *et al.*, 2010).

## Results and discussion

A summary of the results obtained are shown in Table 1. Its analysis highlights the fact that *N. ceranae* was identified in 51% of the studied apiaries across the country, ranging from 19% (in the southern 'Faro' district) up to 89% (in the northern 'Aveiro' district). Despite the fact that considerable district variation in *N. ceranae* prevalence levels were found throughout mainland Portugal, no biologically meaningful geographical pattern (either associated with north/south, inland/coastal or altitudinal transects) was observed. *N. apis* infected samples were not encountered in this study (Figure 1).

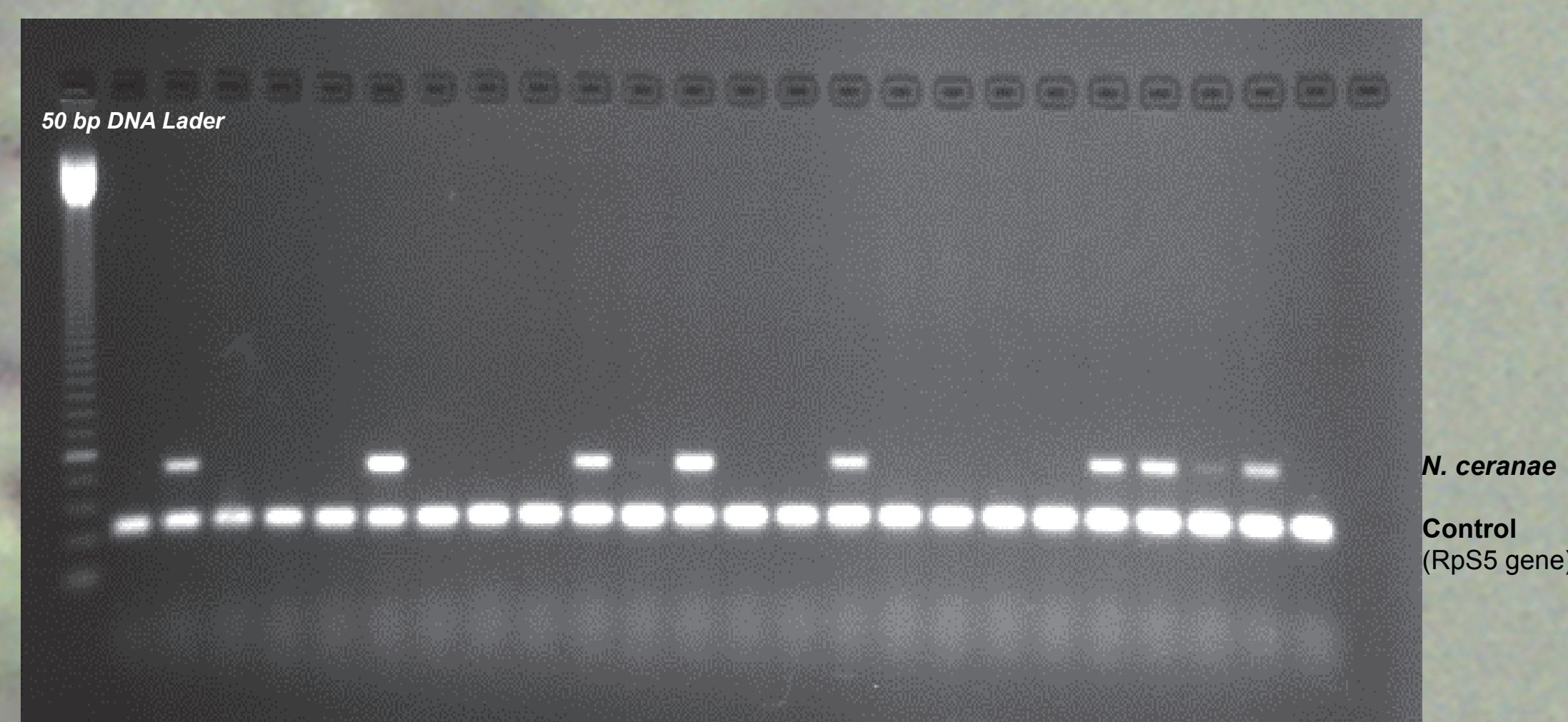


Figure 1- Agarose gel (2%) stained with ethidium bromide, showing 24 lanes PCR products

Table 1- Prevalence of *Nosema ceranae* in Portugal

Districts	Nº of Apiaries	Positive to <i>N. ceranae</i>	%Positive <i>N. ceranae</i>
Aveiro	9	8	89%
Beja	23	11	48%
Braga	12	4	33%
Bragança	16	10	63%
Castelo Branco	14	7	50%
Coimbra	14	7	50%
Évora	21	11	52%
Faro	21	4	19%
Guarda	9	6	67%
Leiria	13	9	69%
Lisboa	16	6	38%
Portalegre	23	14	61%
Porto	14	12	86%
Santarém	16	7	44%
Setúbal	17	5	29%
Viana do Castelo	13	6	46%
Vila Real	13	9	69%
Viseu	13	5	38%
Mean	15	8	51%

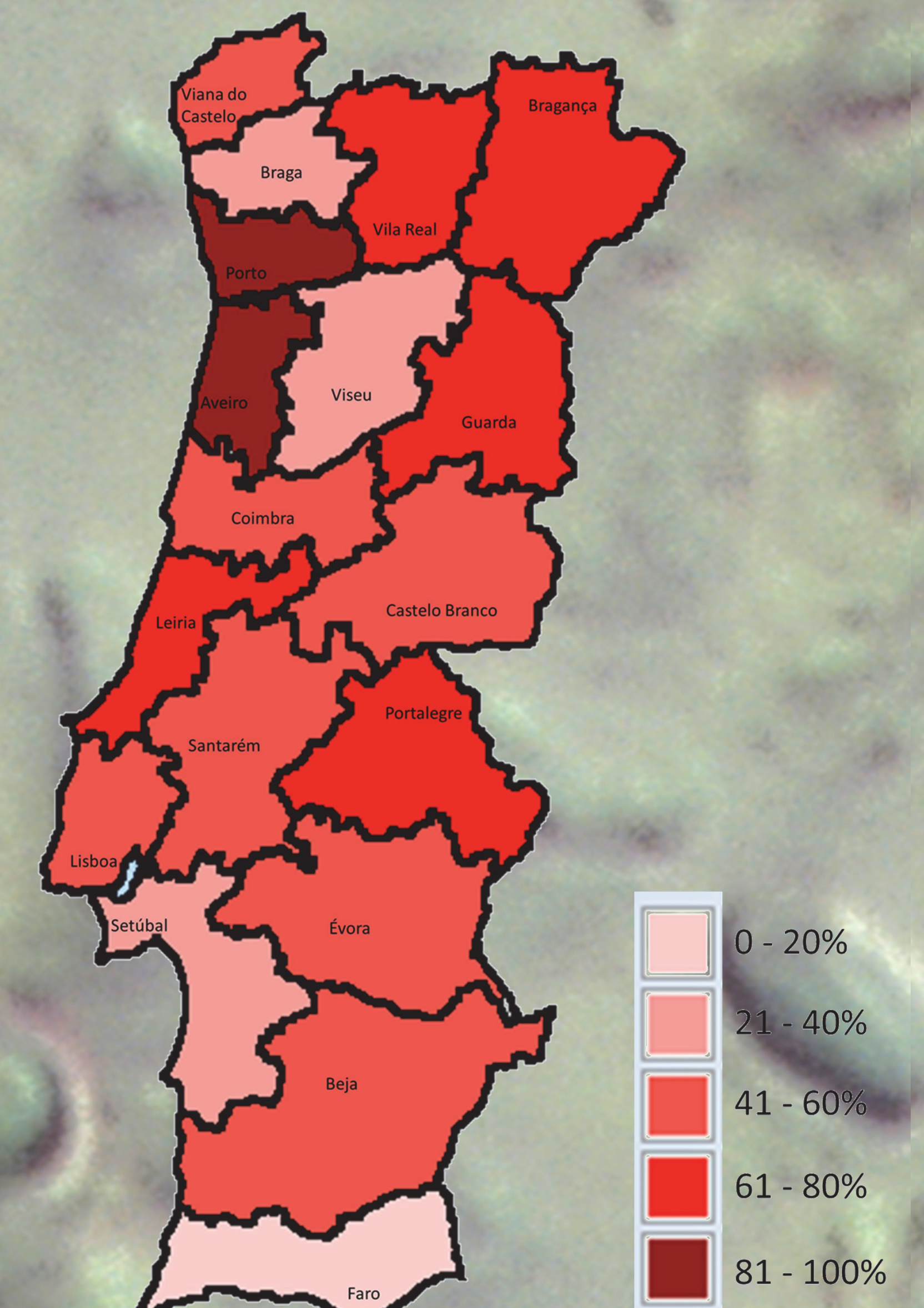


Figure 2- Prevalence of *N. ceranae* in Portugal

## Conclusions

In our work, only *N. ceranae* was identified. It is also acknowledged that *N. ceranae* was present in apiaries from all districts of mainland Portugal, confirming its high adaptability to different geographical / climatic conditions and beekeeping contexts.

## References

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