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DISTRIBUTION OF THE *NOSEMA CERANAE* (MICROSPORA, NOSEMATIDAE) IN THE APIARIES IN UKRAINE

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Distribution of *Nosema ceranae* (Microspora, Nosematidae) in the Apiaries in Ukraine. Odnosum, H. V. — Investigated the distribution of microsporidia *Nosema ceranae* — the pathogen of so-called «Asian» *Nosema* disease in the apiaries of Ukraine. Investigated 784 samples of bee`s podmore in a large extent affected by Nosemosis, that have been sent by beekeepers for research from 11 regions of Ukraine (Kyiv, Poltava, Vinnytsia, Chernihiv, Zhytomyr, Sumy, Zaporizhia, Donetsk, Volyn, Lviv, Khmelnytsky) in 2014–2016. Microsporidia *Nosema ceranae* was found in 74.5 % of the analyzed bee samples, i. e. on prevalence it dominated the microsporidia *Nosema apis.* Clinical signs and course of *Nosema* disease in bee families, where been found microsporidia *Nosema ceranae*, were classic, namely the weak development of families in the spring, in some families — the presence of traces of diarrhea.
Key words: *Nosema ceranae, Nosema apis*, *Nosema disease*, microsporidia, *Apis mellifera* L., bee families, apiaries, beekeeping, distribution.

Introduction

Microsporidia (or Microspora) — a phylum of an obligate intracellular protozoan parasites — eukaryotes. The subject of the article is not a discussion about relation of microsporidia to the protozoa or the fungi, which is recently so popular (Issi, 1986; Karpov, 1990; Westheide, Rieger, 1996; Sokolova, Issi, 2001; Franzen, 2008; Scherbak, Tsarychkova, 2008; Corradi, Keeling, 2009; Soo Chan et al., 2010). We focus on distribution and economic significance of entomopathogenic microsporidia, parasitizing *Apis mellifera* (Linnaeus, 1758), which has a major role in the pollination of the most entomophilous cultures.

The disease, which causes by microsporidia in honeybees and which is widespread in areas of intensive beekeeping, called nosemosis. This parasitic disease causes periodic mass deaths in the bee colonies not only in Ukraine but all over the world (Grobov et al., 1987; Bourgeois et al., 2009; Pacini et al., 2016). Until recently, the agent of nosemosis of honey bees considered to be the microsporidia *Nosema apis* (Zander, 1909). It has been shown quite recently that in many countries the nosema disease is caused not only by *Nosema apis*, but also by *Nosema ceranae* Fries, Feng, Silva, Slemenda & Pieniazek, 1996 (Fries et al., 1996; Martin-Hernandez et al., 2007; Roudel et al., 2013; Csaki et al., 2015).

Nosema ceranae was originally described as a parasite of the Asian honey bee *Apis cerana* (Fabricius, 1793) (Fries et al., 1996), and the nosemosis it caused was called "Asian". Later *Nosema ceranae* was discovered also in European honey bees in all the continents (Klee et al., 2007; Fries, 2010; Branchiccela et al., 2013; Bollan et al., 2013), and in some countries it was found to be either the only agent of the nosemosis, or prevailing *Nosema apis* in distribution. *Nosema ceranae* prevails in apiaries in most states of the USA (Chen et al., 2009), South America (Mendoza et al., 2016), the Balkan countries (Stefanovic et al., 2010), and Japan (Yoshiyama, Kimura, 2011), Canada (Williams et al., 2008), Turkey (Rahsan et al., 2016), Iran (Nabian et al., 2011). Distribution of microsporidia *Nosema ceranae* on apiaries has been studied already in most EU countries (Klee et al., 2007; Kasprzak, Topolska, 2007; Bacandritsos et al., 2010; Stefanovich et al., 2010; Bollan et al., 2013).

Nosema ceranae has been recorded by Tokarev et al. (2010), Zinatullina et al. (2011), and Ignatieva et al. (2012) from Russian Federation, where it dominates over *Nosema apis* in the southern regions. Distribution of *Nosema ceranae* in Estonia, Latvia, Lithuania, Belarus, Moldova, most regions of Russia, Georgia, Armenia, Azerbaijan, Kazakhstan, Uzbekistan, and Kyrgyzstan, as well as in many other countries, remained unknown.

In Ukraine, *Nosema ceranae* was found and recorded by the author with collaborators in 2013 in the apiaries of Kyiv, Poltava, and Zaporizhzhya Regions (Yefimenko et al., 2014). However, actual distribution of this pest species in Ukraine stayed unknown.

This study aimed to study distribution of *Nosema ceranae* in the apiaries over Ukraine; for this, we took samples of live and dead bees from the apiaries in different regions of Ukraine, where nosemosis is recorded, examined the condition of bee families and clinical signs of the nosemosis, and analyzed the samples of bees under light microscope to detect invasion by *Nosema ceranae* and *Nosema apis.*

Material and methods

Samples of dead bees were collected in apiaries in 2014–2016 in Donetsk (Dobropilla, 2 apiaries, 80 samples) and Kyiv Regions (Skvyrsa District, Kryvoshyyintsi, 3 apiaries, 60 samples) in 2014, Kyiv Region (Boryspil District, Chubinsky, 1 apiary, 40 samples) in 2015, Zaporizhzhya (Vilniansk, Melitopol, 1 apiary, 64 samples), Sumy (Glukhiv, 2 apiaries of Krolivets, 3 apiaries, 115 samples), Poltava (Orzhytsia and Hrebinka, 2 apiaries, 52 samples), Chernihiv (Nizhyn, 3 apiaries, 67 samples), Vinnytsia (Pohrebysche, 1 apiary, Illincy, 1 apiary, 88 samples), Khmelnytskyi (Vinkivtsi settlement, 2 apiaries, 29 samples), Zhytomyr (Ruzhin and Baranivka 2 apiaries, 67 samples), Volyn (Lutsk, 1 apiary, 34 samples), from of Lviv Region (Zolochiv, Drohobych, and Sambir, 3 apiaries, 48 samples), and of Kyiv Regions (Kyiv-Svyatoshinsky District, Bucha, 1 apiary, 40 samples) in 2016. Total 784 bee samples were analyzed.

Microscopic analysis and the rate of bees invaded by the microsporidia spores were determined by conventional methods. Bee abdomens are separated and placed in a porcelain mortar, thoroughly triturated with the addition of distilled water at a rate of 1ml per abdomen, and homogenized. A drop of the prepared suspension applied on a glass slide, covered with a coverslip, and examined under the microscope at a middle magnification (×400–600) in a shaded field of the view, examining at least 20 fields of the view (Voronin, Issy, 1974; Grobov et al., 1987).

Nosema apis spores are oval, size varies 4.5–7.5 x 2.5–3.5 μm, covered by a cover 0.2–0.3 μm (Grobov, 1987). *Nosema ceranae* spores straight, oval, slightly curved, size varies 3.6–5.5 x 2.3–3.0 μm and the thickness of the cover is about 0.137–0.183 μm (Fries et al., 1996).

Morphometric analysis was carried out by microscoping of the drop of suspension of spores in the bright field of the light microscope. As a standard were used samples of *Nosema apis* and *Nosema ceranae* spores, species belonging of which was set by the methods of the light microscopy and molecular phylogeny (Giles, 2008; Tokarev et al., 2010; Zinatullina et al., 2011; Ignatieva et al., 2012) (fig. 1).

Results

Spores of microsporidia were found in all analyzed samples of bees, with 10–500 spores per field of the view (magnification ×400). Morphometric analysis showed the accordance of size characteristics of identified spores (length, width, length-to-width and shape) to the indicators specific to *Nosema ceranae*. As statistically reliable distinction of dimensional

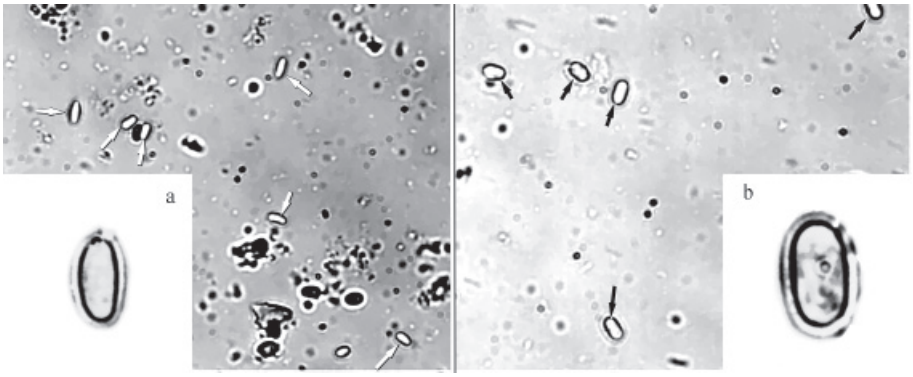

Fig. 1. *Nosema ceranae* (a) and *Nosema apis* (b) under the light microscope (×400 and ×1000) (after Giles (2008), with changes).

characteristics of morphotypes, spores which belong to *Nosema apis* and *Nosema ceranae*, were established by a method of sequencing of ribosomal RNA gene region, so the revealed spores we identified as *Nosema ceranae*.

Of the 784 samples, 305 samples (38.9 %) contained *Nosema ceranae* microsporidia, 200 samples (25.5 %) — *Nosema apis* microsporidia; 279 samples (35.6 %) were invaded by both microsporidia species, i. e., *Nosema ceranae* was present in 74.5 % of the analyzed samples of bees affected by nosemosis.

Nosema ceranae spores were found in 11 regions of Ukraine (fig. 2).

Of the 784 analyzed bee samples only in 81 samples (10.3 %) were observed signs of diarrhea, which with the same frequency observed in those samples where it was found *Nosema apis*, and in those where been observed *Nosema ceranae*, or where they were met together. In other words, this feature was not characteristic for any one type of microsporidia. A characteristic feature at mono- and mixed invasion on condition of strong invasion degree was a weak development of bee colonies.

Discussion

The presence of *Nosema ceranae* spores in 74.5 % of the analyzed samples of bees from 11 regions of Ukraine clearly shows that this species is widespread in the apiaries of Ukraine in different climatic zones and significantly prevails *Nosema apis*.

The nosemosis clinic in examined apiaries was classic, namely the weak development of families in the spring, with tracks of diarrhea in some of them. Diarrhea is not always a characteristic feature of nosemosis caused both by *Nosema apis* (Grobov et al., 1987; Efimenko, 2000) and *Nosema ceranae*. Its pathogenesis largely depends on the degree of bee's contamination by these parasites, either microsporidia *Nosema apis* or *Nosema ceranae* (Pavlichenko et al., 2012; Yefimenko et al., 2013).

Microsporidia do not have their own mitochondria and live exceptionally due to the ATP produced the host, so manifestation of disease occurs usually in the spring, in the period of the high energy loss, which is connected with the beginning of growing of the

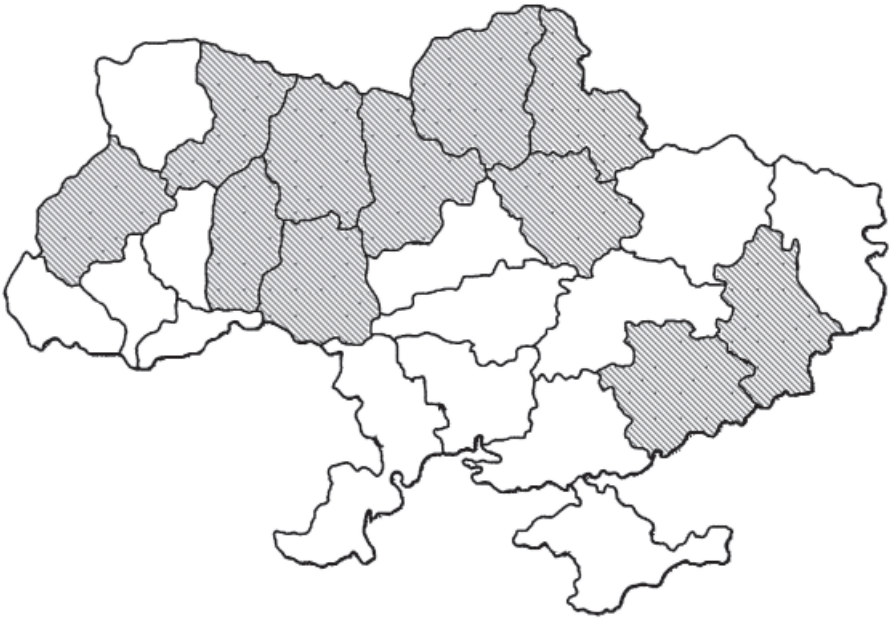

Fig. 2. Regions of Ukraine, where *Nosema ceranae* spores were found.

brood and need to maintain an optimal climate in the nest (Yefimenko et al., 1994). Such a nosemosis manifestation we observed in the apiaries, where both *Nosema ceranae* and *Nosema apis* were detected. We observed the manifestation of disease in the rest of the year, mostly in autumn, is extremely rare and provoked by a high percentage of varroosis, intoxication by honeydew etc. Besides, it provokes nosemosis exacerbation, which may cause mass death of bees, presence of pesticides in food, weak bee families, unable to support the microclimate in the nest, late feeding bees by sugar syrup.

Conclusion

1. For the first time on the territory of eleven regions of Ukraine, which are situated in different climatic zones, was studied the dissemination of microsporidia *Nosema ceranae* — bee's Nosemosis pathogen, which is also called "Asian".

2. Microsporidia *Nosema ceranae* was detected in the samples of bees from 11 regions of Ukraine, where been received the samples of bees for analysis. In some apiaries *Nosema ceranae* met along with microsporidia *Nosema apis* and very few samples of bees, where been presented only microsporidia *Nosema apis*.

3. Clinical signs and manifestation of Nosema disease in bee families from which were selected samples and revealed microsporidia *Nosema ceranae*, were classic, namely weak development of families in the spring, in some families — the presence of traces of diarrhea.

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References

Bacandritsos, N., Granato, A., Budge, G., Papanastasiou, I., Roinioti, E., Caldon, M., Falcaro, C., Gallina, A., Mutinelli, F. 2010. Sudden deaths and colony population decline in Greek honey bee colonies. *Journal of Invertebrate Pathology,* **105** (3), 335–340.

Bollan, K., Hothersall, J., Moffat, C., Durkacz, J., Saranzewa, N., Wright, G., Raine, N., Highet, F., Connolly, C. 2013. The microsporidian parasites *Nosema ceranae* and *Nosema apis* are widespread in honeybee (*Apis mellifera*) colonies across Scotland. *Parasitology Research,* **112** (2), 751–759.

Bourgeois, A. L., Rinderer, T. E., Beaman, L. D., Danka, R. G. 2009. Genetic detection and quantification of *Nosema apis* and *Nosema ceranae* in the honey bee. *Journal of Invertebrate Pathology,* **103** (1), 53–58.

Branchiccela, M., Zunino, P., Antunez, K., Martin-Hernandez, R., Higes, M. 2013. Genetic Diversity of *Nosema ceranae* Assesed by Inter Sequence Simple Repetition (ISSR) Technigue. *XXXXIII International Apicultural Congress, 28 September–4 October 2013, Kyiv, Ukraine,* 193.

Chen, Y., Evans, J., Zhou, L., Boncristiani, H., Kimura, K., Xiao, T., Litkowski, A., Pettis, J. 2009. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. *Journal of Invertebrate Pathology*, **101** (3), 204–209.

Corradi, N., Keeling, P. 2009. Microsporidia: a journey through radical taxonomical revisions. *Fungal Biology Reviews,* 23, 1–8.

Csáki, T., Heltai, M., Markolt, F., Kovács, B., Békési, L., Ladányi, M., Péntek-Zakar, E., Meana, A., Botías, C., Martín-Hernández, R., Higes, M. 2015. Permanent prevalence of *Nosema ceranae* in honey bees (*Apis mellifera*) in Hungary. *Acta Veterinaria Hungarica,* **63**(3), 358–369.

Efimenko, T. M. 2000. What we know about *Nosema disease* of honey bees and measures for its prevention. *Pasika,* 2, 20–21 [In Ukrainian].

Franzen, C. 2008. Microsporidia: a review of 150 years of research. *The Open Parasitology Journal,* 2, 1–34.

Fries, I., Feng, F., Silva, A., Slemenda, S., Pieniazek, N. 1996. *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *European Journal of Protistology*, 32, 356–365.

Fries, I. 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). *Journal of Invertebrate Pathology,* **103** (1), 73–79.

Giles, B. 2008. What is *Nosema ceranae* and how do you test for it? *Bee Craft*, 1, 7–8.

Grobov, O. F., Smirnov, A. M., Popov, E. T. 1987. *Diseases and pests of honeybees: Handbook.* Agropromizdat, Moscow, 1–334 [In Russian].

Ignatieva, A. N., Zinatullina, Z. A., Tokarev, Y. S. 2012. The spread of pathogens honeybee *Nosema i*n the European part of Russia. *Materials of the International Youth Conference "Infectious diseases of arthropods", St. Petersburg, Pushkin, 25–29 March,* 24–27.

Issi, I. V. 1986. Microsporidia as a phylum of parasitic protozoa. *Microsporidia. Protozoology*, 6–136 [In Russian].

Karpov, S. A. 1990. *System of Protista*. OmPI, Omsk, 1–75 [In Russian].

Kasprzak, S., Topolska, G. 2007. Nosema ceranae (Eukaryota: Fungi: Microsporea) — a new parasite of western honey bee *Apis mellifera* L. *Wiadomosci Parazytologiczne*, **53** (4), 281–284.

Klee, J., Besana, A., Genersch, E., Gisder, S., Nanetti, A., Tam, D., Chinh, T., Puerta, F., Ruz, J., Kryger, P., Message, D., Hatjina, F., Korpela, S., Fries, I., Paxton, R. 2007. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 96, 1–10.

Martin-Hernández, R., Meana, A., Prieto, L., Salvador, A., Garrido-Bailón, E., Higes, M. 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Applied and Environmental Microbiology*, 73, 6331–6338.

Mendoza, Y., Diaz-Cetti, S., Ramallo, G., Santos, E., Porrini, M., Invernizzi, C. 2016. *Nosema ceranae* Winter Control: Study of the Effectiveness of Different Fumagillin Treatments and Consequences on the Strength of Honey Bee (Hymenoptera: Apidae) Colonies. *Journal of Economic Entomology*, 1–228.

Nabian, S., Ahmadi, K., Nazem, S., Gerami, A. 2011. First Detection of *Nosema ceranae*, a Microsporidian Protozoa of European Honeybees (*Apis mellifera*) in Iran. *Iranian Journal of Parasitology*, **6** (3), 89–95.

Pacini, A., Mira, A., Molineri, A., Giacobino, A., Bulacio Cagnolo, N., Aignasse, A., Zago, L., Izaguirre, M., Merke, J., Orellano, E., Bertozzi, E., Pietronave, H., Russo, R., Scannapieco, A., Lanzavecchia, S., Schnittger, L., Signorini, M. 2016. Distribution and prevalence of *Nosema apis* and *N. ceranae* in temperate and subtropical eco-regions of Argentina. *Journal of Invertebrate Pathology*, 141, 34–37.

Pavlichenko (Odnosum), H., Yefimenko, T. 2012. The lifespan of bees, depending on the dose of infection by microsporidia *Nosema apis* Zander. *Materials of the International Youth Conference "Infectious diseases of arthropods", St. Petersburg, Pushkin, 25–29 March*, 50–51 [In Russian].

Rahsan, I., Devrim, O., Ayhan, G., Olgay, K. 2016. Does *Nosema ceranae* Wipe Out *Nosema apis* in Turkey. *Iranian Journal of Parasitology,* **11** (2), 259–264.

Roudel, M., Aufauvre, J., Delbac, F., Blot, N. 2013. New Insights On The Genetic Diversity Of the Honeybee Parasite *Nosema cranae* Based On the Multilocus Sequence Analysis. *XXXXIII International Apicultural Congress, 28 September–4 October 2013,Kyiv, Ukraine,* 193–194.

Scherbak, G. I., Tsarichkova, D. B. 2008. Zoology of Invertebrates. *Kyivskyi Universytet Publishing and Printing Centre*, Kyiv, 1–640.

Sokolova, Yu. Ya., Issi, I. V. 2001. The insect protists and features of their pathogenesis. *In*: Glupov V. V., ed. *Insect pathogens: structural and functional aspects*. Kruglyi stol, Moscow, 4, 116–117 [In Russian].

Soo Chan, L., Nicolas, C., Sylvia, D., Fred, S., Patrick, J., Joseph, H. 2010. Evolution of the sex-Related Locus and Genomic Features Shared in Microsporidia and Fungi. *PLoS One,* **5** (5), https://doi.org/10.1371/journal.pone.0010539

Stevanovic, J., Stanimirovic, Z., Genersch, E., Kovacevic, S., Ljubenkovic, J., Radakovic, M., Aleksic, N. 2010. Dominance of *Nosema ceranae* in honey bees in the Balkan countries in the absence of symptoms of colony collapse disorder. *Apidologie*, 42, 49–58.

Tokarev, Y. S., Ignatieva, A. N., Zinatullina, Z. A. 2010. Molecular diagnostics of *Nosema*. *Beekeeping*, 5, 18–19.

Voronin, V. N., Issy, I. V. 1974. About the methods of working with microsporidia. *Parasitologia*, 3, 272–273 [In Russian].

Westheide, W., Rieger, R. (Hrsg.) 1996. Spezielle Zoologie. Erster Teil: Einzeller und Wirbellose Tiere. Gustav Fischer Verlag, *Stuttgart,* 1–909.

Williams, G., Shafer, A., Rogers, R., Shutler, D., Stewart, D. 2008. First detection of *Nosema ceranae,* a microsporidian parasite of European honey bees (*Apis mellifera*), in Canada and central USA. *Journal of Invertebrate Pathology*, **97** (2), 9–192.

Yefimenko, T., Bodnarchuk, L. 1994. Some properties of host parasite interactions beetween honey bees from different generations and their microsporidial parasite *Nosema apis. 12-th Congress of the International Union for the Study of Social Insects IUSSI, Paris, Sorbonne, 21–27 August*, 348.

Yefimenko, T., Halatyuk, O., Pavlichenko (Odnosum), H. 2013. Influence of invasive burden of *Nosema apis* on the dynamics of bees mortality. *Materialy z 50 naukowa Konferencia pszczelarska, Pulawy, 16–18 kwietnia*, 69–71.

Yefimenko, T., Odnosum, H., Tokarev, Y., Ignatieva, A. 2014. *Nosema ceranae* Fries et al., 1996 (Microspora, Nosematidae) — a honey bee parasite in Ukraine. *Ukrayinskyy Entomolohichnyy Zhurnal*, 2 (9), 71–76.

Yoshiyama, M., Kimura, K. 2011. Distribution of *Nosema ceranae* in the European honeybee, *Apis mellifera* in Japan. *Journal of Invertebrate Pathology*, **106** (2), 263–267.
Zander, E. 1909. Tierische Parasiten als Krankheitserreger bei der Biene. *Leipziger Bienenzig*, 24, 147–150.
Zinatullina, Z. A., Ignatieva, A. N., Zhigileva, O. N., Tokarev, Y. S. 2011. „Asian" *Nosema* in Russia. *Beekeeping*, 10, 24–26.

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