



VIRUSES AND PARASITIC DISEASES IDENTIFICATION BY MOLECULAR METHODS OF THE HONEY BEE (*APIS MELLIFERA*)

P. HRISTOV¹, A. GEORGIEVA², D. SIRAKOVA¹, B. NEOV¹ & G. RADOSLAVOV¹

¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria; ²Institute of Experimental Morphology, Pathology and Anthropology with Museum, Sofia, Bulgaria

Summary

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A lot of factors lead to dramatic diminution of bee populations, reduction of bee productivity and thus disruption of the process of pollination of farming crops and wild plants. Among such factors are known diseases of economic significance affecting bees and their brood – viral, bacterial (European and American foulbrood) fungal (chalkbrood and stonebrood) and parasitic (varroosis and noseiosis). To the present moment at least 19 viruses infecting honey bee are known, most important among which are the Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Deforming wing virus (DWV), Cloudy wing virus (CWV), Black queen cell virus (BQCV), Chronic bee paralysis virus (CBPV) and Israeli acute paralysis virus (IAPV). It is believed that their most important vectors are the ecto- (Varroa) and endoparasites (Nosema) of bees. Global data demonstrate that mainly 3 viruses are connected with significant bee colony losses – CWV, ABPV and IAPV. The first one was initially connected to Colony Collapse Disorder (CCD), but at a later stage it was concluded that the reasons for CCD are more complex. In Europe ABPV and IAPV are indicated as one of the reasons for the reduction of bee families in post winter season. No data are available about the extent of the spread of viral pathogens in bee colonies nor about the presence of co-infections in bee colonies in Bulgaria. Samples of 50 bee colonies from 3 regions of Bulgaria were tested for infection with 6 types of viruses (ABPV, KBV, DWV, BQCV, CBPV, IAPV) and the two related endoparasitic vectors *N. apis* and *N. ceranae*. Methods of the investigation include both DNA and RNA isolation, conventional and multiplex PCR (Nosema) and Reverse Transcriptase PCR (RT-PCR) (Viruses). All amplified DNA fragments of viruses were sequenced and analysed and data were deposited in GenBank. The results of the analysis showed a high infection of beehives with only one invasive species -*N. ceranae* (over 70%). Four virus types were detected - CBPV (7/15%), DWV (4/9%), ABPV (2/4%) and BQCV (1/2%). We have established a correlation between viral and noseiosis infections in over 90% of the beehives. Sequence analysis of viruses shows their affiliation to new types strains, which is an indicator of their possible local origin. The detection and the determination of the genetic diversity, virulence and the pathological effects of parasitic, viral, fungal and bacterial honey bee diseases by means of molecular methods in Bulgaria will contribute to a wide spectrum of monitoring, diagnosis and management of these diseases. *Funding: This work was supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Sofia [grant number 06/10 17.12.2016].*