In partnership with

9th COLOSS Conference Book of Abstracts

Kyiv, Ukraine, 27-29 SEPTEMBER 2013

(prior to the XXXXIII Apimondia International Apicultural Congress 29 September - 04 October 2013)

9th COLOSS Conference

Topic

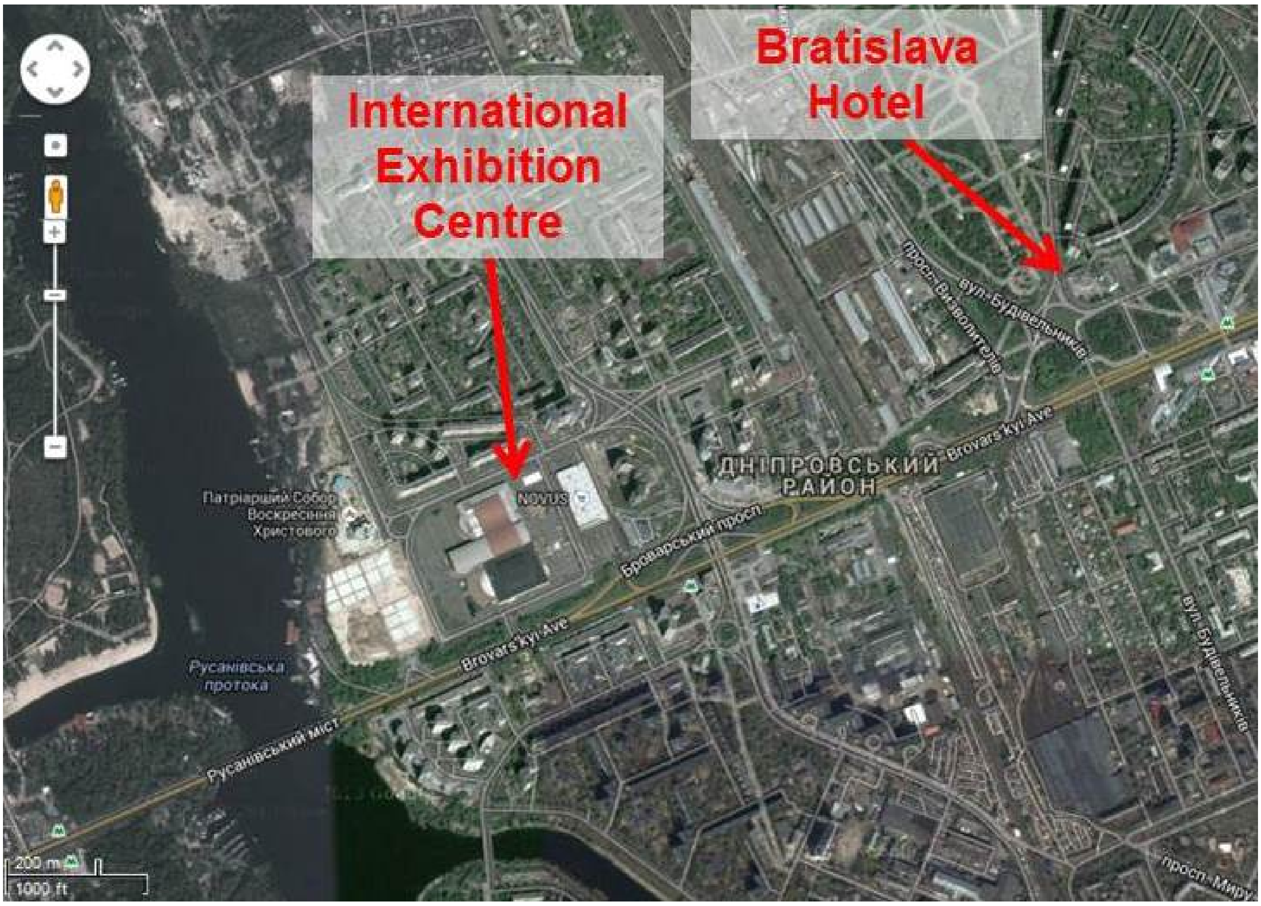
- International meeting of COLOSS, providing an update on the network's achievements and future directions, **including formalizing COLOSS as a legal entity, introduction of constitution and by-laws, and election of network administrators**.

When

- Friday, 27 September – Evening for current Executive Committee members (Hotel Bratislava)
- Saturday, 28 September – Full day for all (Hotel Bratislava)Sunday, 29 September – Morning for all (Hotel Bratislava)

Where

- Hotel Bratislava (http://www.bratislava.com.ua/?lang=en), Kyiv, Ukraine



Registration

- Registration fee: **20 € payable on site**.
- Registration for Apimondia is separate from COLOSS and can be accomplished at http://apimondia2013.org.ua.

Travel & Accommodation

- For travel information and accommodation details, please refer to the Apimondia website (http://apimondia2013.org.ua/en/)

ORGANIZER CONTACTS FOR FURTHER INFORMATION	
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Agenda

Friday, 27 September 2013

Time		Location
19:00-20:00	Current Executive Committee meeting	Hotel Bratislava, Red Room

Saturday, 28 September 2013

Time	Session 1 – Adoption of COLOSS Statutes and EC Election	Location
8:30-9:00	**Sign-in**	Hotel Bratislava, Red Room
9:00-9:05	Welcome and organizational matters by P Neumann & G Williams	
9:05-10:30	Adoption of statutes and election of new Executive Committee by F Hatjina	
10:30-11:00	**Break**, with drinks & snacks	
Session 2 – COLOSS Updates chaired by G. Williams		
11:00-11:10	COLOSS past achievements by P Neumann	
11:10-11:20	The COLOSS BEEBOOK by V. Dietemann	
11:20-11:30	Working Group 1 overview by chairs	
11:30-11:40	Working Group 2 overview by chairs	
11:40-11:50	Working Group 3 overview by chairs	
11:50-12:00	Working Group 4 overview by chairs	
12:00-13:00	**Lunch & poster set-up**	
13:00-13:10	Announcement of newly-elected Executive Committee members by F Hatjina	
Session 3 – Honey Bee Health chaired by P. Neumann		
13:10-13:20	Carreck *et al*.- Progress on breeding hygienic bees at the University of Sussex	
13:20-13:30	Chauzat *et al*. - A pan-European surveillance program on honeybee colony losses	
13:30-13:40	Hou *et al*. (Chejanovsky) - Fate of the Israeli acute paralysis virus in CCD colonies	
13:40-13:50	Forsi *et al*. - The occurrence of *Acarapis externus* mite in honey bee colonies in an apiary in Iran	
13:50-14:00	Giacomelli *et al*. - Honey bee queen caging: the artificial absence of brood as a strategy for varroatosis containment	
14:00-14:10	Milbrath *et al*. (Huang) - *Nosema* infection method affects honey bee survival	
14:10-14:20	**Speed Break**	
14:20-14:30	Hatjina *et al*. (Kryger) - A review of methods for assessing honey bee queen quality characters used in some European countries	
14:30-14:40	Nasr *et al*. - Honey bee health in Alberta, Canada: present state and economic costs	
14:40-14:50	Nikolenko *et al*. - The problem of increased honey bee mortality in Russia	
14:50-15:00	Özkırım *et al*. - The role of natural and chemical disinfectants using for hygenic beekeeping on microflora and pathogenic agents of honeybees	
15:00-15:10	Pernal *et al*. - Proteomic marker-assisted selection for resistance to American foulbrood	
15:10-15:20	Pettis - Factors affecting queen health	

Saturday, 28 September 2013 continued

Session 4 – Posters		
15:30-17:00	Poster session with drinks & snacks	Hotel Bratislava, Red Room
20:00-	Social dinner and student award presentation (not covered by COLOSS)	Hotel Bratislava

Sunday, 29 September 2013

Time	Session 5 – Executive Committee Meeting 1	Location
8:30-9:15	Executive Committee Meeting 1 (election of President & Vice-President, *etc.*)	Hotel Bratislava, Alliance Hall
Session 6 – Future COLOSS Networking chaired by newly-elected President		
9:30-9:40	van der Steen *et al*. - The honey bee and the metal pollution of the environment	
9:40-9:50	Gregorc - Networking for laboratory and large scale field tests	
9:50-10:00	Hatjina *et al*. - Field assessment of impacts of different neonicotinoids on honey bee queens and drones	
10:00-10:10	van der Steen & Brodschneider - C.S.I. Pollen – Citizen Scientist Investigation on pollen diversity forage available to honey bees	
10:10-10:30	**Break**, with drinks & snacks	
Session 7 – General Assembly Round Table Discussions chaired by newly-elected President		
10:30-11:30	General Assembly round table discussion on COLOSS affairs, including establishment of new Working Groups, *etc.*, chaired by newly elected President	
Session 8 – Executive Committee Meeting 2		
11:45-13:00	Executive Committee Meeting 2 (formation of committees, *etc.*)	

Submitted Poster Line Up

1. Abdi *et al*. - First report of DWV in Tunisia
2. Adjlane *et al*. - Distribution and prevalence of pathogens in honey bee colonies *Apis mellifera intermissa* in Algeria: results of 2012
3. Albaba - Diversity of vegetation and pollen collected by honeybees in the West Bank-Palestine
4. Anido *et al*. (Antúnez) - Causes of colony losses in Uruguay, a country without massive colony losses
5. Aziz *et al.* (El-Niweiri) - Survey of *Nosema apis* (ZANDER), *Acarapis woodi* (RENNIE), and Varroa destructor in Sudan
6. Brodschneider *et al*. - Winter colony losses in Austria in 2012/13
7. Brusbardis - COLOSS survey results in Latvia
8. Chaimanee *et al*. (Chantawannakul) - Cross infections of *Nosema ceranae* in *Apis cerana* and *A. mellifera*
9. Mookhploy *et al*. (Chantawannakul) - Detection and phylogenetic analysis of honey bee viruses in Asian honey bees and European honey bees in northern Thailand
10. Chlebo *et al*. - Genetical structure of bee population in Slovakia
11. Gajda *et al*. - *Nosema ceranae* has been present in honey bee colonies in Poland at least since 1994 and appears to have ousted *Nosema apis*
12. Gray *et al*. - Colony loss rates from the 2013 Scottish monitoring survey and a comparison of conditions for carrying out surveys internationally
13. Ivanova *et al*. - Allozyme and microsatellite DNA polymorphism in *Apis mellifera* L. from Bulgaria and genetic markers, usable for discrimination of local Bulgarian honey bees
14. Kezic *et al.* - Packaged bees market - definitions and standardization
15. Loewe *et al*. - Protein and carbohydrate share in two temporal castes of a honey bee colony
16. Malagnini *et al.* (Angeli) - Imidacloprid treatments of apple orchards lead to residues and chronic toxicity in bee colonies
17. Nauen *et al*. (Tritschler) - Optimization of *Varroa* treatment by formic acid application
18. Porrini *et al*. (Mutinelli) - BEENET, a network for monitoring honey bee mortality and colony losses in Italy
19. Rajper - Genetic diversity based on morphometric analysis of honey bee population of Khairpur (Pakistan) in a global context

20. Ruiz *et al*. - SWOT Analysis of B^3 (Biomonitoring of Bees as Bioindicators)

21. Saltykova *et al*. - The uncontrolled use of neonicotinoids is a threat to world beekeeping

22. Santrac *et al.* - Coloss questionnaire is a good tool in monitoring honey bee losses

23. Soroker *et al*. - Disruption of *Varroa destructor* honeybee chemosensing, for future *Varroa* management.

Submitted Registration Forms

	Last & First Name	Country
1	Abdi, Khaoula	Tunisia
2	Adjlane, Noureddine	Algeria
3	Albaba, Imadeddin	Palestine
4	Angeli, Sergio	Italy
5	Antúnez, Karina	Uruguay
6	Bienkowska, Malgorzata	Poland
7	Bogo, Gherardo	Italy
8	Bortolotti, Laura	Italy
9	Bouga, Maria	Greece
10	Brodschneider, Robert	Austria
11	Brusbardis, Valters	Latvia
12	Büchler, Ralph	Germany
13	Carreck, Norman	UK
14	Carvalho, Stephan	Brazil
15	Carvalho, César	Brazil
16	Chantawannakul, Panuwan	Thailand
17	Chauzat, Marie-Pierre	France
18	Chejanovsky, Nor	Israel
19	Chlebo, Róbert	Slovakia
20	Costa, Cecilia	Italy
21	Crailsheim, Karl	Austria
22	Dahle, Bjørn	Norway
23	de Miranda, Joachim	Sweden
24	Dietemann, Vincent	Switzerland
25	Drazic, Maja	Croatia
26	Ellis, Jamie	USA
27	El-Niweiri, Mogbel	Sudan
28	Filipi, Janja	Croatia
29	Formato, Giovanni	Italy
30	Forsgren, Eva	Sweden
31	Forsi, Mohammad	Iran
32	Francis, Roy Mathew	Denmark
33	Gajda, Anna	Poland
34	Giacomelli, Alessandra	Italy
35	Gray, Alison	UK
36	Gregorc, Aleš	Slovenia
37	Grzęda, Urszula	Poland
38	Hatjina, Fani	Greece
39	Huang, Zachary	USA
40	Ibrahim, Yasser	Egypt
41	Ivanova, Evgeniya N.	Bulgaria
42	Janeš, Mateja	Croatia
43	Kezic, Nikola	Croatia
44	Kretavicius, Justinas	Lithuania
45	Kristiansen, Preben	Sweden

	Last & First Name	Country
46	Kryger, Per	Denmark
47	Loewe, Anika	Austria
48	Mayr, Josef	Austria
49	Medrzycki, Piotr	Italy
50	Mladenović, Miča	Serbia
51	Mortensen, Ashley	USA
52	Mutinelli, Franco	Italy
53	Nasr, Medhat	Canada
54	Neumann, Peter	Switzerland
55	Nikolenko, Alexey	Russia
56	Özkirim, Asli	Turkey
57	Pernal, Stephen	Canada
58	Pettis, Jeff	USA
59	Prdjun, Sasa	Croatia
60	Rajper, Naheed	Pakistan
61	Rašić, Slađan	Serbia
62	Ritter, Wolfgang	Germany
63	Rogers, Richard E.L. (Dick)	USA
64	Ruiz, José Antonio	Spain
65	Saltykova, Elena	Russia
66	Santrac, Violeta	Bosnia/Hercegovina
67	Schiesser, Aygün	Turkey
68	Schmehl, Daniel	USA
69	Soroker, Victoria	Israel
70	Tanner, Gina	Switzerland
71	Teixeira, Erica	Brazil
72	Topolska, Grażyna	Poland
73	Tosi, Simone	Italy
74	Tritschler, Manuel	Germany
75	van der Steen, Jozef	Netherlands
76	van der Zee, Romée	Netherlands
77	vanEngelsdorp, Dennis	USA
78	Vejsnæs, Flemming	Denmark
79	Wilde, Jerzy	Poland
80	Wilkins, Selwyn	UK
81	Williams, Geoff	Switzerland
82	Yañez, Orlando	Switzerland

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Submitted Oral Presentations

Submitted Poster Presentations

Progress on breeding hygienic bees at the University of Sussex.

Norman L Carreck[1,2], Karin L Alton[1], Gianluigi Bigio[1], Luciano Scandian[1], Hasan Al-Toufailia[1], and Francis L W Ratnieks[1].

[1]Department of Biological and Environmental Science, University of Sussex, Falmer, Brighton, East Sussex, BN1 9QG, UK.
[2]International Bee Research Association, 16, North Road, Cardiff, CF10 3DY, UK.

Hygienic behaviour in honey bees is a naturally occurring genetic trait, meaning that it can be selected for using conventional bee breeding methods. Our aim since 2008 has been to selectively breed and then test under UK field conditions, a strain of hygienic honey bees, and to then make this available to UK beekeepers. Any bee breeding programme is open ended. We have now, however, reached the stage of being able to evaluate the progress that we have made so far. Despite the difficult conditions for queen rearing in 2012, in the autumn, we were able to pass a number of our reared queens to various members of the Bee Farmers Association in different parts of the UK for evaluation under a range of conditions. A second strain of bees bred locally by a semi commercial beekeeper and selected for resistance to chalkbrood for many years has also been tested for hygienic behaviour and incorporated in our breeding programme. The lack of a well organised queen rearing industry in the UK will however, prove a limiting factor to any attempt to improve the genetic potential of our bees. As part of our programme at LASI of outreach to stakeholders such as beekeepers, over the last two seasons we have therefore run several successful workshops at the University to introduce the techniques for measuring hygienic behaviour, and these have been well received and were attended by beekeepers travelling from a considerable distance. We hope that these workshops will result in beekeepers around the country following our lead and developing local breeding groups to make the best use of their locally occurring bees.

A pan-European surveillance program on honeybee colony losses

Chauzat Marie-Pierre[1, 2], Garin Emmanuel[2], Saugeon Cécile.[1], Hendrikx Pascal.[2], Ribière Magali[1]

[1] Anses, Sophia Antipolis Laboratory, Bee Disease Unit, European Union Reference Laboratory for bee health, BP 111, 06902 Sophia Antipolis, France
[2] Anses, Maisons-Alfort Laboratory for Animal Health, Epidemiological Surveillance Unit, 94706 Maisons-Alfort, France

In 2009, the EFSA project entitled "Bee mortality and bee surveillance in Europe" underlined the general weakness of most surveillance systems in Europe. Data on colony losses were not representative at country level and were not comparable at EU level due to the lack of standardisation and harmonisation.

In 2011, following the decision to set up a standardized European wide surveillance programme, the European Commission required the technical help from the European Union Reference Laboratory for bee health that was appointed to the ANSES Sophia-Antipolis laboratory in February 2011. A standard protocol has been applied in 17 Member States implying common sampling methods implemented in all countries, common visit forms used in all apiaries and the training of the bee inspectors.

During this study, the main criteria to be assessed are the colony losses (overwintering colony mortality and seasonal mortality). The major honeybee diseases should also be surveyed by the clinical observations of varroosis, American foulbrood, European foulbrood, nosemosis, and chronic paralysis. The *Varroa destructor* infestation rate before winter should be assessed on living bees. Finally, this project should ensure an early alert in case of the detection of the two exotic arthropods, *Aethina tumida* and *Tropilaelaps* spp. mites.

The first visit was performed between mid July 2012 (Finland) and mid December 2012 (Spain). More than 3 000 apiaries and beekeepers actually took part to the surveillance (participation rate of 97.2 %). More than 35 000 samples were collected during the visit to date. A web based database has been developed to allow collecting and saving all the data for all participants from their countries.

The COLOSS *BEEBOOK*: capitalising on worldwide honey bee research through method standardisation

V. Dietemann [a,b,*], J Ellis [c], P Neumann [b,d]

[a] Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, Bern, Switzerland;
[b] Social Insect Research Group, Zoology and Entomology Department, University of Pretoria, Private Bag X20 Hatfield, 0028 Pretoria, South Africa
[c] Department of Entomology and Nematology, University of Florida, Bldg. 970 Natural Area Dr., P.O. Box 110620, Gainesville, FL 32607-0620, USA
[d] Institute for Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

COLOSS (prevention of honey bee COlony LOSSes) is a global network made up of ~300 members from 63 countries. Its members aim to improve the well-being of honey bees through collaborative networking and project development. During their activities, COLOSS members recognised the need for research methods to be standardised to facilitate comparison of experimental results gathered from different countries. The COLOSS "*BEEBOOK*: standard methods for *Apis mellifera* research" will be the definitive, yet evolving, honey bee research manual. It is composed of 29 peer-reviewed chapters authored by more than 200 of the world's leading honey bee experts. Chapters describe methods for studying honey bee biology, methods for understanding honey bee pests and pathogens, and methods for breeding honey bees, among others. It is published both online as two Open Access Special Issues in the *Journal of Apicultural Research* and as a hard copy book for use at the laboratory bench. Due to the fast evolving field, it will be kept updated via an online tool and subsequent new editions.

This communication presents the structure and content of the *BEEBOOK*.

The Occurrence of Acarapis externus Mite in honey bee colonies in an apiary in Iran

Mohammad Forsi[1], Ghojooghi Rahim[2]

[1]Iran Veterinary Organization, Tehran, Iran
[2]Iran Veterinary Organization, Gogan, Iran

Acarapis dorsalis and A. externus are external parasitic mites that consume the hemolymph of honey bees (Root, 1990). Morphologically they are difficult to differentiate from one another and from A. woodi, even at high magnification (Bailey and Ball, 1991). It is unclear whether they contribute to the general weakness of a colony, or if they prey preferentially on weak colonies (Jillson 2007).

They are not considered a pest of honey bees as there have not been any reports of either of these mites causing visible symptoms or injury to bees, or the presence of these external mites affecting colony performance.

In an apiary in north of Iran, we observed a severe infestation of colonies to Acarapis externus.

The parasites in **all** life **stages** (**egg**, **larva**, nymph, and **adult**) were observed on honeybees. Most of the colonies were perished after suffering from severe losses of population.

The microscopic videos of Acarapis externus in infested bees and also the videos of apiary and colonies have been prepared for presenting.

Honey bee queen caging: the artificial absence of brood as a strategy for varroatosis containment

Alessandra Giacomelli, Marco Pietropaoli, Marcella Milito, Martina Pizzariello, Francesco Scholl, Giovanni Formato

Beekeeping Unit, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Via Appia Nuova 1411, 00178 Rome, Italy

For several years Italian beekeepers use to fight varroatosis, that represents a prior topic in modern beekeeping, an adequate period of queen caging to obtain a useful brood absence. This technique allows to enhance the miticide efficacy of different low environmental impact compounds administered to the colonies. From 2008, the Beekeeping Unit of the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana realized various field trials in different periods of the year to determine the acaricide efficacy of the queen caging. In addition, different devices have been tested to evaluate the best strategies to cage the queen and allow her welfare, monitoring also if the queen oviposition during the caging period can modify the wellness of the whole colony.

Networking for Laboratory and large scale field tests

Aleš Gregorc

Agricultural Institute of Slovenia

Environmental pollution and pesticides can influence adults and larvae and effects can be detected also on colony level. Some of the chemicals may enrich in the bee's body and affect organs and tissues. Studies at all levels should be considered and evaluated using different research methods and organizational structures. Networking for possible collaborative experimental work will be discussed.

A review of methods for assessing honey bee queen quality characters used in some European countries

Fani Hatjina[1], Malgorzata Bieńkowska[2], Leonidas Charistos[1], Robert Chlebo[3], Cecilia Costa[4], Maja Dražić[5], Janja Filipi[6], Aleš Gregorc[7], Evgeniya Neshova Ivanova[8], Nikola Kezic[9], Jan Kopernicky[10], Per Kryger[11], Marco Lodesani[4], Vesna Lokar[7], Mica Mladenovic[12], Beata Panasiuk[2], Plamen Pavlov Petrov[13], Slađan Rašić[12], Maja Ivana Smodis Skerl[7], Flemming Vejsnæs[14], Jerzy Wilde[15]

[1]Hellenic Institute of Apiculture, Hellenic Agricultural Organization 'DEMETER" Nea Moudania, Greece
[2]Research Institute of Horticulture, Apiculture Division in Pulawy, Poland
[3] Slovak University of Agriculture in Nitra, Slovakia
[4] Agricultural Research Council, Honey bee and Silkworm Research Unit, Bologna, Italy
[5] Croatian Agricultural Agency, Zagreb, Croatia
[6] The Univesity of Applied Sciences Marko Marulic in Knin, Croatia
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[9] Faculty of Agriculture University of Zagreb, Zagreb, Croatia
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[11] Institute of Agroecology, Aarhus University, Denmark
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[13] Agricultural University, Plovdiv, Bulgaria
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[15] Apiculture Division University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,

Quality is a term that when used in relation to queens and drones infers to certain quantitative physical and/or behavioural characters such as: heavy live weight, high number of ovarioles, large size of spermatheca, high number of spermatozoa in spermatheca, and be free from diseases and pests. However, it is also known that the performance of a honey bee colony is the result of its queen's function as well as of the drones mated with her. These two approaches are run together quite often and give a general picture of the queen production and selection. Here we have described the most common and well known anatomical, physiological, behavioural and performance characters related to the queens: such as the live weight of the virgin queen, the live weight of the laying queen , the diameter and volume of spermatheca, the number of ovarioles the weight of ovaries the number of spermatozoa in spermatheca, the brood pattern, the egg laying ability/ fecundity the brood production the colony population development , the honey production, the hygienic behaviour, the defence behaviour, the calmness/ sitting on the comb, the swarming We also have reported new finding related to the insemination procedure and we had an inside view of the sanitary conditions of the colony. This is the first step to summarize this type of diverse data for such an important issue.

Field assessment of impacts of different neonicotinoids on honey bee queens and drones

Co-ordinator: Fani Hatjina

Hellenic Institute of Apiculture, Hellenic Agricultural Organization 'DEMETER" Nea Moudania, Greece

The aims of the suggested project are: To determine the effect of particular neonics on life span of queens; To measure the effect on the egg laying capacity of the queens; To detect any abnormalities on the brood development; To measure sperm quantity and viability on mature drones. A pilot study focusing in imidacloprid has been performed in 2013 and a more detailed study will be performed in 2014. Imidacloprid will be given to the colonies by means of sugar solution and pollen patty in doses of 5ppb and 200ppb. Egg laying ability of the queens and sperm viability will be accessed every 21 days. Development of the eggs will be also accessed 9 days after each colony measurement. Feeding will last for almost 3 months and virus analysis will be performed on worker and drones before and after the feeding starts. Chemical analysis on stored beebread and honey will also be performed. The colonies will have young queens and their lifespan will be recorder. Virus analysis will also be performed on the queens at the end of the experiment.

Fate of the Israeli acute paralysis virus in CCD colonies

Chunsheng Hou[1], Hadassah Rivkin[1], Yossi Slabezki[2] and Nor Chejanovsky[1]

[1]Entomology Department, Institute of Plant Protection, The Volcani Center, Israel. [2]Beekeeping Division, Extension Service, Israeli Ministry of Agriculture. (ninar@volcani.agri.gov.il)

Certain honey bee viral pathogens in general, and the Israeli acute paralysis virus (IAPV) in particular, were associated with the striking collapse of honey bee colonies termed CCD. We identified a group of colonies in Israel matching the hallmarks of CCD. These colonies, highly positive for IAPV, were rescued and the fate of these virus was followed for one year by molecular methods. Periodic examination of the IAPV status indicated that the viral titers were decreasing from winter to summer. However, IAPV was able to replicate and conserved its infectious potential anytime tested. Our study suggests that in CCD colonies rescued before total collapse, IAPV maintains an active replicative form that, given favorable conditions, enables its rapid amplification.

Nosema infection method affects honey bee survival

Meghan O. Milbrath[b] Xianbing Xie[a,b] Zachary Y. Huang[b]

[a]Department of Laboratory Animal Science, 71 East Yangming Road, Nanchang University, Nanchang, Jiangxi 330006, China
[b]Department of Entomology, 243 Natural Science, Michigan State University, East Lansing, MI 48824, USA

Nosema ceranae infection can reduce survival of the Western honey bee, Apis mellifera, but experiments examining its virulence have highly variable results. This variation may arise from differences in experimental techniques. We examined survival effects of two techniques: Nosema infection at day 1 without anesthesia and infection at day 5 using CO_2 anesthesia. All bees infected with the latter method had poorer survival. Interestingly, these bees also had significantly fewer spores than bees infected without anesthesia. These results indicate that differences in Nosema ceranae-induced mortality in honey bees may be partially due to differences in experimental techniques.

Honey Bee Health in Alberta, Canada: Present State and Economic Costs

Medhat Nasr[1], Samantha Muirhead[1] and Emmanuel Laate[2]

1. Crop Research and Extension Division,
2. Economics and competitiveness Division,

Alberta Agriculture and Rural Development, Edmonton, Alberta, Canada

The apiculture industry is a vital component of the agricultural industry of Alberta, Canada. Currently Alberta has 282,000 bee colonies (43% of the bees in Canada). Honey production in Alberta was estimated at $60 million in 2012. In Alberta over 80,000 bee colonies are annually rented for pollinating hybrid canola to ensure high yield of canola seeds for growers. The estimated contribution of honey bees to the pollination of certified hybrid canola for seed production is over $200 million with an estimated value chain of $600 million per year.

Honeybees are currently experiencing a battery of threats including bacterial brood diseases, *Nosema spp*, viruses, parasitic mites, and more recently, the mysterious colony collapse disorder (CCD). Since 2007 a surveillance program has been established to determine annual colony mortality, possible causes of bee mortality and management practices used by commercial beekeepers with 400 or more honey bee colonies. Provided information by this subset of professional commercial beekeepers would be useful in giving a better understanding of bee colony mortalities.

The results of the annual survey for the past 6 years will be discussed. Honey bee colony mortalities have been decreasing from 30% per year (2007-2009) to 15%- 20% (2010-2013). Results of the management practices' survey and the bee colony and sample examinations showed that significant causes of elevated high colony mortalities were varroa mites and nosema. The failure of treatments of these two pests is generally associated with high colony mortalities. Harsh winter conditions and cold spring were also contributing factors supported by regional differences within Alberta. The ambiguity surrounding colony mortalities in relation to CCD in Alberta, Canada was removed. Overall in spite of reported high colony mortalities in the first three years from 2007-2009, beekeepers were able to replace all dead colonies and increase their numbers from 224,000 in 2008 to 282,000 in 2012.

An economic study to estimate the potential economic losses for the Alberta beekeeping Industry due to reported high bee colony kill was conducted. The estimated loss of income from honey sales and pollination fees, replacement of dead colonies and additional labor costs ranged from $16-25 million per year in 2007-2009. Recent estimated costs of economic losses per 1000 colonies will be discussed.

The problem of increase the honey bee mortality in Russia

A.G. Nikolenko, E.S. Saltykova, L.R. Gaifullina, R.A. Ilyasov,
A.V. Poskryakov, S.A. Fakhretdinova

Institute of Biochemistry and Genetics, Ufa Science Centre RAS, Ufa, Russia

In the 1990s in Russian the norm of colony losses was 10%. Now It is much higher, although the exact details are still missing. The closed hive in long winter 5-7 months adds difficulty in causes identifying of the colony death. A few Russian researchers work with separate issues (analysis of the gene pool of the bees, the study of new pests and pathogens, the risk assessment of new pesticides, the search for the causes of increased mortality bees), but to create a complete picture requires the efforts of a much larger of qualified researchers.

Gene geographic analysis allowed us to identify remaining local populations of Apis mellifera mellifera L. in Urals, and make a detailed analysis of the structure and genetic processes in one of them (Burzyan population). Obviously, crossbreeding of subspecies from different evolutionary branches and the subsequent outbreeding depression is one of the main causes of colonies mortality increase in Russia. Many unsolved problems remain in the areas of intraspecific taxonomy of the honey bee.

We have received also interesting data about the high risk of neonicotinoids, the second important cause of increased mortality of bees in Russia. The imidacloprid single action even at concentrations 1 ppm causing a rapid and intense bowel disorder, creating favorable conditions for the expansion of pathogens. Thus, the neonicotinoids may be one of bee immunodeficiency syndrome reasons even in full compliance with all environmental regulations of their application.

Molecular monitoring of pathogens showed the widespread presence of Nosema apis in the Urals, as well as the high incidence of Ascosphaera apis in worker bees in visually healthy families (up to 50% of families).

Finally, on the basis of fractions of chitosans we have developed preparation of new generation - an adaptogen (an immunomodulator) for the honeybee. Our Laboratory of biochemistry of adaptability of insects is dealing with these issues since 1996, and we look forward to any form of cooperation.

The role of natural and chemıcal dısınfectants usıng for hygenıc beekeepıng on mıcroflora and pathogenıc agents of honeybees

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Apicultural economic development strongly relies on the health status of honey bee colonies. The normal physiology of honey bees always receives much less attention than medicine and veterinary science. The growth of microorganisms inside honey bees is an important factor in bee health. These organisms, called commensals, grow in the honey bee and constitute normal flora which can compete with pathogens, preventing the colonization of these pathogens. By this respect keeping bees healthy may be more important than treating colonies. In apiaries, honey bees usually contact with the surfaces of combs and hives during their activities. They could carry all pathogens from outside, nectar, pollen sources or other colonies. Disinfection of hives is very important to prevent colonies from pathogens. Especially in spring and autumn seasons, honey bee colonies revise themselves and colony growth is started. During both seasons several methods are used for cleaning hives by beekeepers. In this study, some natural ways of disinfection (like fire or blowtorch and sunshine as UV disinfection) and natural disinfectant products extracted from botanical sources are compared with chemical disinfectants (like KClO and hydrogen peroxide) or nanotechnological ones (like quaternary amine-containing organosilicon salt (Si-QAC) for their effects on microflora of honeybees and hives. The experiments set up in laboratory (in vitro) and in field (in vivo) conditions. By microbiological and PCR methods, the dynamic of microflora and the presence of pathogens are determined in terms of using different kinds of disinfectants. The results showed that all kinds of disinfectants are useable to prevent colonies from pathogens. On the other hand, duration of disinfectant in hives is the main factor for the disruption of honey bee microflora. For this reason, natural disinfectant products should be preferred for both prevention from pathogens and also protect the indigenous microflora in hives.

Proteomic Marker-Assisted Selection for Resistance to American Foulbrood

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A primary goal of our project was to evaluate the use of proteomic marker-assisted selection for enhancing disease resistance in honey bee populations. In 2011, 622 colonies were tested for hygienic behaviour (HB) across four Canadian provinces. A portion of these colonies was then randomly selected to establish an unselected benchmark population (*n*=83) while an F_0 population was established (*n*=110) from colonies most highly expressing HB. We successively tested, selected and propagated two generations from our F_0 during 2012 and produced a third generation in 2013 using: (1) a freeze-killed brood assay and (2) a panel of proteomic markers, as the selection criteria. This constituted a direct comparison of proteomic-based marker-assisted selection (MAS) against traditional field-based phenotypic selection (FAS) on HB.

The first generation from our selectively-bred stocks (FAS, MAS), as well as the benchmark population and an imported stock, was challenged with local isolates of *Paenibacillus larvae*. This was accomplished by introducing American foulbrood (AFB)-infected comb to colonies headed by the selected queens. In the first generation, the selected FAS stock had significantly greater hygienic behaviour scores ($\bar{x}$ = 88%) than benchmark ($\bar{x}$ = 71%) or imported queens ($\bar{x}$=61%), with MAS queens ($\bar{x}$ = 77%) being intermediate between the two.

Colonies were assessed biweekly for visible symptoms of AFB, and adult bee samples were analysed for *P. larvae* spores, *Nosema* spp. spores, and the presence of *Varroa destructor*. Despite having equivalent *P. larvae* spore loads at two weeks post-inoculation, colonies selected for hygienic behaviour using the freeze-killed brood assay (FAS) had a significantly reduced incidence of clinical symptoms of AFB at 12 weeks (15%) than did the benchmark population (63%) or imported stock (100%), whereas the protein marker-assisted selected stock (MAS) was intermediate (40%) between the FAS and benchmark stocks. Further evidence of improved colony-level resistance to AFB for our FAS and MAS selections compared with other stocks was based on reduced numbers of colony-forming units of *P. larvae* cultivated from broodnest workers using diagnostic media. Honey production and levels of *V. destructor* and *Nosema* spp. did not vary among all stocks in the F_1 generation. To our knowledge, this is the first time that proteomic markers have been used to guide a selective breeding program in agriculture. Evaluations of the F_3 continue in 2013.

Factors affecting queen health

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In honeybees, queen health is vital to the survival of the colony. In many parts of the world queens are failing or being superseded after as little as six months instead of the 1-2 year life span that is often reported. To explore possible causes of queen failure we dissected queens from failing colonies, including drone laying queens and measured mating success and sperm viability. Additionally, we treated healthy queens with various low doses of pesticides, based on the amounts found in adult bees and wax, and then measured sperm viability. We found that many drone laying queens still had sperm present but at a mortality rate above normal. Similarly, we found that queens from failing colonies had high sperm mortality compared to queens in healthy colonies. Lastly, when we artificially treated queens with sub-lethal dosages of pesticides we could kill up to 50% of sperm in only 7 days.

The honey bee and the metal pollution of the environment

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One of the by-catches of the foraging trips of the honey bee is the atmospheric deposition of metals (particulate matter) from anthropogenic sources and soil erosion. Within the foraging area of the honey bee colony of approximately 7 – 12 km^2, additionally to pollen, particles < 50 µm will stick to the bee's body. Most of the particles in the branched hairs of the bee will be removed by auto grooming but a considerable number of particles is left and indicates the atmospheric deposition of pollutants. However not all metals detected in the bee, are the result of atmospheric deposition. The bee has a spectrum of metals in the body, resulting from metals in the larval feed and from pollen. A number of these metals have biological functions as elemental part of proteins. Significant increases of metal concentrations are considered as a result of an overexposure to particles containing metals and revealing metal pollution. The spatial and temporal variation of metal in honeybee, the link to metal concentration in the ambient air and to specific land uses is presented.

C.S.I. Pollen – Citizen Scientist Investigation on Pollen Diversity Forage available to Honey Bees

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Honey bees depend for their protein, fat and mineral resources completely on pollen. As some pollen might lack one essential amino acid, pollen diversity is crucial for a complete supply with essential amino acids. The decrease of pollen diversity, e.g. due to large scaled agricultural monocultures, is believed to be a threat for apiculture and a possible factor in the honey bee and wild bee decline. Although recognized as a threat, the link between land use and low pollen diversity for honey bees is poorly documented. Therefore at the Coloss WG3 workshop in Bled 2012, the initiative was taken to form an European Citizen Scientist (CS) group of beekeepers that collect pollen on a regular base in the course of the bee season. In a first approach, the CS's determine the diversity of pollen collected by honey bees based on pollen pellet color following a strict protocol. This is submitted online to a central database together with supplementary data as the exact location of the apiary (using Google maps GPS function) and a simple characterization of the habitat within 2 km around the apiary. In 2013 the project started with a pilot in the Netherlands and other countries joined in the course of the season. The project will run till end of 2015 and can be prolonged. This first approach allows the rough identification of seasonal and regional pollen availability. In a facultative second approach, palynological analysis of pollen samples from selected CS can be conducted to validate or correct the field approach of pollen color discrimination. The project will provide insight in the link between pollen diversity, landscape and land use which enables focused large scale field studies and mitigation methods. Being involved in this project the CS beekeepers learn about the feed availability for their colonies and develop an awareness of the link between honey bee colony health and the environment and form a community that cooperates in practical large scaled honey bee studies. We will present first insights learned from the pilot studies regarding the importance of communication with Citizen Scientists to standardize sampling and also the first results obtained. Each country is responsible to organize and minister its own group of Citizen Scientists. We welcome all countries to join and make this the first and largest project investigating pollen diversity with the help of Citizen Scientists and their honey bee colonies throughout Europe.

First report of DWV in TUNISIA

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Deformed wing virus (DWV), an affliction of honey bees (*Apis mellifera Intermissa*), is an important threat to honey bee health. It's a positive-single-stranded RNA virus in the order Picornavirales that has been found in many places across the globe.

The presence of honey bee viruses has never been rigorously identified in Tunisia apiaries. We thus conducted a preliminary molecular survey study of DWV in 50 samples of working adult bees from four northern governorates in Tunisia (Beja, Jendouba, Bizerte and Seliana) during October-December 2011 to understand and monitor the spread of this virus.

Using a one-step real-time RT-PCR (SYBR Green) and Sanger sequencing, we found the first evidence of DWV. The virus was detected in 34% of samples from four northern governorates in Tunisia, mainly in Beja with an increase in the prevalence during the late fall and early winter season.

Kea words: honey bees, Deformed wing virus, one-step real-time, prevalence.

Distribution and prevalence of pathogens in honey bee colonies Apis mellifera intermissa in Algeria: results of 2012

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The objective of this study was to determine the prevalence and saisonalty of the most important diseases of honeybees in honey bee located in the north of Algeria. (one of the important Beekeeping centre in Algeria). Pathogenic agent in this study sought are *Varroa destrutor*, *Nosema* sp, *Paenibacillus larvae* and *Ascosphera fungi*. A total, 56 apiaries were visited and 123 bee samples were analyzed. the sampling was conducted on the beekeeping in the three different seasons of spring, summer and fall in the year 2012. The results showed a wide variation in the presence of these diseases in the local bee colonies *Apis mellifera intermissa*. 41% of samples were contaminated with *Nosema* sp. Aspcosphera apis is present in 18% of the apiaries analyzed with a peak in early spring. The appearance of this disease is favored by a sudden drop in temperature and high humidity conditions recorded at the beginning of spring. 35% of the colonies are characterized by the presence of *Paenibacillus larvae*. American foulbrood is a serious disease of honey bees that inflicts considerable economic losses on beekeepers in Algeria. 100% of the samples are infested with Varroa destructor infestation rate of adult bees which varies between 3 and 12%.

Climatic conditions and practices of beekeepers negative influence on the distribution of these pathogens. A strong correlation was found between the rate of infestation of *Varroa* and the presence of Nosema and *Aspcosphera in* honey bee. However, more intensive studies covering different regions and apiaries are required to gain further insights into the prevalence and severity of pathologies in Algeria in two races of local honey bee *Apis mellifera intermissa* et *sahariensis.*

Diversity of vegetation and pollen collected by honeybees in the West Bank-Palestine

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The nectar and pollen, are the major sources for the fulfillment of the nutritional demands of honeybees. The contents of these sources are vary based on the floral diversity, seasonality. And flowering phenology. Palestine is a small country rich in biodiversity, due to Palestine's location at the crossroads of three bio-geographical (Mediterranean, Desert and Steppe) and botanic regions of various climatic conditions, topography, geomorphology, geology and soils. This endows the Palestinian Territories with a rich variety of plant life including some 2780-plant species (Feinbrun- Dothan and Danin, 1991).

The present study aims at surveying the diversity of honeybees foraging plant species. Thesurvey was conducted through bi-weekly visit to the selected sites located in some reserves agricultural fields, randomly selected of the southern part of the West Bank-Palestine.

Four (4) linetransects of 1,000m in length each, were selected and stationed on two separate points on each site at every study visit. The start and end of each transect were marked with the National flags made to enhance visibility. Moving on the transects, each and every 5 steps. A total number of 393 species of plants were identified belonging to 57 families by the end of the study.

Causes of colony losses in Uruguay, a country without massive colony losses

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In Uruguay, a South American country, massive honeybee colony losses had not been reported so far, and the number of managed colonies has remained stable during the last ten years. However, a decline in honey production and a rise in winter colony losses has been noticed by beekeepers. The aim of the present work was to analyze the distribution and dynamics of the most important honeybee pathogens in Uruguay and to evaluate the causes of those losses.

Varroa destructor resulted the main problem of the colonies, with a prevalence of 76% in March (autumn). The infection significantly decreased after acaricide treatment. BQCV also showed a wide distribution, high prevalence (87%) and it remained high along the whole year. Other viruses (ABPV, DWV and SBV) presented lower prevalences and were restricted to the most important beekeeping areas. The only microsporidium detected was *Nosema ceranae*, with a prevalence of 15 %. *Paenibacillus larvae* and *Acarapis woodi* presented extremely low prevalences, while *Nosema apis*, KBV and IAPV were not detected. Sixteen % of the colonies under study died along the year. Those colonies presented the highest level of *V. destructor* infestation in March and *N. ceranae* in September, than healthy colonies, suggesting that both pathogens are involved in adult bee losses in Uruguay.

Considering that the most important bee pathogens are present and widely distributed in our country, why colony losses are not massive in Uruguay? Possible factors are discussed.

Survey of *Nosema apis* (ZANDER), *Acarapis woodi* (RENNIE) ,and Varroa destructor in Sudan

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Honeybee of Sudan is claimed to be free of the most dangerous parasites and diseases of honeybee. However, the repeated importation of honeybees may spread novel diseases that lead to the eradication of non-adapted regional wild populations. Therefore the present study aimed to survey the occurrence and the infestation level of *Nosema apis* (ZANDER), *Acarapis woodi* (RENNIE) and *Varroa destructor* in Sudan. The study was conducted during the period 2012 and 213. A total of 43 colonies were chosen randomly from location where imported honeybees were repeated introduced particularly Khartoum and Sinnar States. Inspection of honeybees was done by using washing technique for *Varroa destructer* and light microscope to *Nosema apis* and *Acarapis woodi* .Fortunately all the inspected colonies were found to be free of *Nosema spp* and *Acarapis woodi* (RENNIE). However the parasitic mite *Varroa destructor* was detected in 41.8 % of the inspected colonies and 1.68% of the inspected honeybees. The infestation percentage with *Varroa* mite was found to be higher in Sinnar state (64.7%) comparing to Khartoum state (26.9%). High infestation level of *Varroa* mite was found in the imported colonies comparing to wild ones. Likewise there was strong correlation between *Varroa* infestation and the presence of imported honeybee. These findings obviously demonstrated the responsibility of imported bees in spreading *Varroa* mite in Sudan. On the other hand the result suggested that either Sudan is free from Nosema and Acarine diseases or honeybees of Sudan are resistant to these diseases.

Winter colony losses in Austria in 2012/13

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After considerable high colony losses (25.9%) during the winter of 2011/12, we continued our survey using the COLOSS questionnaire the 6th year in a row. We therefore used a mixed media approach (internet, beekeeping journal, meetings) and beekeepers could answer on the internet or on paper. We did not ask for name or contact, but encouraged participants to do so on a voluntary basis. Until the date of survey completion we received answers from 996 beekeepers collectively managing 19524 colonies. Overall colony loss rate in Austria was 17.3% (95% confidence interval: 16.1-18.6%) with marked regional differences. In our survey, 62.8% of respondents identified themselves and loss of identified versus anonymous respondents did not differ. Also response on paper versus response via internet did not differ. A detailed analysis of risk factors for colony winter losses will be presented and discussed. Of special interest is the effect of timing and type of treatment against the parasitic mite *Varroa destructor* and the forage available to honey bees as these factors contribute to overwinter mortality. We have also submitted our results to be included in the international press release and the joint analysis of risk factors. Our monitoring of winter colony losses of honey bees will be continued next year and special attention will be given in dissemination of results to participants, other beekeepers, the public and media.

COLOSS survey results in Latvia

Valters Brusbardis

Latvian Beekeepers Association

Varroa destructor is considered as the main killer of bee colonies in Latvia despite the fact that mite is present in country already for more than 30 years. Use of pesticides in agriculture, Nosema, American foulbrood, European foulbrood and bad beekeeping practise are also considered as significant factors which cause colony loses in Latvia. Latvia did not have well justified annual data about honey bee loses until this year (2013). In order to change this situation Latvian Beekeepers Association run COLOSS survey in 2013.

Beekeepers were informed about COLOSS questionnaire by internet, local beekeeping magazine and post. Answers from beekeepers were collected by internet and post.

Official institutions estimate that there are more than 4300 beekeepers and 83800 bee colonies in Latvia at the moment. From those 519 beekeepers (12%) participated in the project and filled out questionnaire.

Results of survey shows that colony loses during the winter period of 2012/2013 in Latvia are 19,7%. Respondents wintered 17920 bee colonies (21% of all colonies registered in country) and they lost 3522.

Cross Infections of Nosema ceranae in Apis cerana and A. mellifera

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Nosema ceranae was firstly found in the cavity nesting Asian honey bee, *Apis cerana*. We cross infected *N. ceranae* strains which were isolated from *A. mellifera* and *A. cerana* in both honey bees. Immunity-related enzymes in each honey bee species following *N. ceranae* inoculation were also tested at day six after spore feeding. Our results showed that *N. ceranae* isolated from *A. mellifera* had higher infectivity than the isolate from *A. cerana* in both honey bee species. The infection rate of each *N. ceranae* strain between two honey bees showed no difference. This suggests that *N. ceranae* isolated from *A. mellifera* may be more virulent than *N. ceranae* isolated from original host and genetic markers contributing to the pathogenicity should be further investigated. Bees inoculated with *N. ceranae* isolated from *A. cerana* showed lower expression of phenol oxidase (PO) when compared to bees inoculated with another strain. However, transcript level of phenol oxidase in one experimental cage of *A. mellifera* had significantly up-regulated when inoculated with *N. ceranae*. The trends of glucose dehydrogenase (GLD) and Eater expression in inoculated bees greatly varied between experimental cages.

Genetical structure of bee population in Slovakia

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On the territory of Slovakia, around 250,000 honey bee colonies mainly of the Carniolan race of Carpathian and Alpine ecotypes are kept by more than 15 500 beekeepers (year 2012).

Breeding of Carnica queens is actually (year 2013) performed in 3 breeding and in 28 reproduction queen breeding stations. Morphometrics methods are used to verify subspecies purity. First results of the project to discriminate bee population of all lines of Carniolan bee officially kept in Slovakia using microsatelite markers will be reported in this study.

DNA isolation followed the protocol of NucleoSpin Tissue Isolation Kit (Macherey-Nagel). We used 10 microsatellite markers for evaluation of the population. PCR reaction was based on one multiplex using 10 microsatelite markers by ABI 310 Genetic Analyzer. Structure of population have been evaluated in POWERMARKER 3.23 using allelic frequency data. Principal component analyses have been computed in GENETIX 4.05 software.

***Nosema ceranae* has been present in honey bee colonies in Poland at least since 1994 and appears to have ousted *Nosema apis*.**

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At the Laboratory of Bee Diseases at WULS we determined *Nosema* species in 16 dead bee samples from 1994 using PCR. The samples were collected from the bottom boards of hives, or in dead bee traps placed at the entrances of hives, and stored at -18°C.

We also examined samples collected from apiaries near Olsztyn and Puławy between 2009 and 2011. The apiaries /colonies were created specifically for the GEI Experiment. We examined forager bees or bees from the outer comb of the hive. They were collected in July/August from each colony and examined for the presence of *Nosema apis* and *Nosema ceranae* DNA. Although the level of *Nosema* infection tends to decrease in summer, the consequences on the detection of *Nosema spp.* should have been the same in both years of investigation.

All the historical samples we examined showed the presence of *N. ceranae* either together with *N. apis* (12 samples) or a pure infection (4 samples). Those are the first results which suggest that *N. ceranae* was not a rare species in the middle of the 1990s in Europe.

Out of all GEI colonies which presented mixed (*N. apis*+*N. ceranae*) infections in 2009, in 2010 42% were infected with *N. ceranae* only.

This study not only shows that *N. ceranae* was present in Poland long before the mass bee losses of the 21st century, but also that it tends to oust *N. apis* in honey bee colonies.

Colony loss rates from the 2013 Scottish monitoring survey and a comparison of conditions for carrying out surveys internationally

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In Scotland we have carried out surveys of beekeepers since 2006, using a postal survey and a self-administered questionnaire. Since 2008 we have selected our samples randomly, using with permission the membership records of the Scottish Beekeepers' Association. This year our survey has mostly been conducted online using the LimeSurvey software (http://www.limesurvey.org/), enabling a larger random sample to be processed, supplemented by a smaller postal survey. A sample of 300 participants was selected from 1094 possible participants. Of the selected 300, 218 (73%) had email contact details.

The postal element was used only for those beekeepers who were selected but did not have an email contact address, those whose email failed and could not be corrected, and a few who were contacted by email but who experienced technical problems in accessing the survey. This resulted in 94 questionnaires being posted and 3 sent electronically for postal return. Collection of postal results is ongoing at the time of writing.

Preliminary results, based on 78 complete responses collected so far in LimeSurvey from beekeepers, are that 46 (59%) experienced colony losses over winter 2012/13, and that the winter colony loss rate was 44.3%, estimated using the overall proportional loss rate (van der Zee et al., 2013). This represents 139 colonies lost over the winter of 2012-13 out of a total of 314 being managed in the autumn of 2012. Compared to the previous winter, in which 40% of 89 beekeepers reported losses and the overall loss rate was 15.9% (which was low compared to the previous two surveys), this is an extremely high loss rate. In fact it is the highest loss rate since our surveys began, indicating a very difficult winter for bees. The next highest rate was 30.9% over winter 2009-10.

Beekeeper surveys are carried out differently in different countries. We recently undertook a research study to collect information from 30 countries belonging to COLOSS Working Group 1, in order to compare the regulatory frameworks and conditions for sampling, availability of records to use for sampling frames and sampling approaches used. Some results will be presented both from Scotland and also from this comparative study.

Reference:

van der Zee, R., Gray, A., Holzmann, C., Pisa, L., Brodschneider, R., Chlebo, R., Coffey, M.F., Kence, A., Kristiansen, P., Mutinelli, F., Nguyen, K.B., Noureddine, A., Peterson, M., Soroker, V., Topolska, G., Vejsnaes, F., Wilkins, S. (2013). Standard survey methodology for estimating colony losses and explanatory risk factors in Apis mellifera. Journal of Apicultural Research, to appear.

Allozyme and microsatellite DNA polymorphism in *Apis mellifera* L. from Bulgaria and genetic markers, usable for discrimination of local Bulgarian honey bees

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The genetic variability of honey bee populations from 24 Bulgarian provinces and populations of *A. m. carnica*, *A. m. caucasica*, *A. m. ligustica* and *A. m. macedonica* has been studied using allozymic analysis of six enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 loci and DNA analysis of 9 microsatellite loci (Ac011; A024; A043; A088; Ap226; Ap238; Ap243; Ap249 and Ap256). All of the studied allozymic loci were found to be polymorphic in most of the populations. Four alleles were detected at Mdh-1, Me and Pgm loci and three alleles – at Alp and Hk loci. Est-3 locus was polymorphic with six alleles. All of analyzed nine microsatellite loci were found to be polymorphic in all of the populations with the presence of a total 121 alleles. Thirty five private alleles were observed in 19 of the studied populations. Nei's genetic distance between studied populations was calculated and UPGMA and neighbor-joining phylogenetic trees were constructed. Genetic markers, usable for discrimination of Bulgarian honey bees were described in this study. Some differences between Bulgarian and other *A. m. macedonica* honey bee populations were discussed which suggests that local Bulgarian honey bee could be a different ecotype of *A. m. macedonica*. The data of this research provide new information concerning the genetic variability and differentiation among Bulgarian honey bee populations and would be useful for selection and conservation purposes.

Package bees market- definitions and standardization

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The trade of honey bee queens and package bees is a global phenomenon. A wide range of methods is currently in use in placing queen and package bees on the market. Production techniques, equipment, recommendations and practices differ over the continents. The aim of the activity is to prepare recommendations for the placement on the market of the bees to equally serve as standards for both scientific and practical beekeeping purposes. Benefits and threats (biodiversity, diseases) of the global market need to be discussed. International standards and/or recommendation are intended to ensure safe and reliable transportation and high quality packages/queens for the final beekeeper. These should cover standard equipment (cages for packages, queens), food stores, welfare during transportation, pre-treatment of diseases, existence of the drones in packages, and race certification, with the aim of reducing differences among producers and increasing customer confidence in final product. Other issues such as problems related to transport and race discrimination may be addressed, to provide producers with more efficient tools for the global market. Could discuss formation of the pre-market approval procedures and the post-market surveillance effects of the honeybee trade.

Protein and Carbohydrate Share In Two Temporal Castes Of A Honey Bee Colony

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The food exchange via trophallaxis is a significant social interaction in the complex honey bee society. Nurse bees have a pivotal role in this social system. They are able to discriminate between different larvae (queen, workers and drones) and between differently aged worker larvae and feed each of them with distinct composed diets varying in the amount of protein and carbohydrates. The castes in a honey bee colony are not rigid but change with age polytheism which might also influence the trophallactic interactions. We investigated if the differentiation in feeding is continued among worker bees after emerging as adults. In a lab assay, the trophallactic interactions between a donor nurse bee (7-9 days of age) and two types of recipient bees (one-day old, n=31 and 7-9 days old nurse bees, n=26) were observed. For each experiment a donor bee was caged with one recipient bee in an arena. Each donor bee was fed with 15µl of 3M glucose solution 10min before it was introduced into the arena to evoke offering behavior. The recipients were forced to regurgitate and starved 3h (one day old bees) and 1h (nurse bees) to induce begging behavior before the experiment started. A single trial lasted until the end of one trophallactic contact. After the trial was stopped the recipient and donor bees were dissected to collect their honey stomach content. The honey stomach content was analyzed for its total protein and total carbohydrate content. Our results so far show that there are no significant differences in the onset and duration of trophallaxis between the tested recipient bees ($p > 0.05$ Mann Whitney). During a single trophallactic contact recipient nurse bees received more sugar solution than one-day old bees ($p < 0.05$, Mann Whitney). The analyses of the honey stomach content show that the amount of total carbohydrates and protein found in the complete honey stomach after a single trophallaxis is significantly higher for recipient nurse bees than for one day old bees ($p < 0.05$, Mann Whitney). It seems that recipient nurse bees are more efficient in the food share per time unit than one day old bees. In further studies we will investigate if nurse bees produce permanently secretions from the hypopharyngeal glands and if they swallow these and store them in their honey stomach.

Imidacloprid treatments of apple orchards lead to residues and chronic toxicity in bee colonies

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The neonicotinoids insecticides clothianidin, thiamethoxam and imidacloprid have been found to pose a high risk tobees health and apartial ban of these molecules has been issued by the European Commissionfor a two-year period, starting on December 2013. Since the introduction of neonicotinoids in 1991, these insecticides have been linked to the declining bee populations across the globe, registered especially in the northern Hemisphere. At present there is a growing body of evidence that neonicotinoids have a detrimental effect on several insect pollinators. The major concerns have regarded their use as seed treatments (particularly on corn, canola, sunflower and soybean) and the contaminated dust during planting. In 2008 and in 2011 large bee mortality was associated with contaminated dust during corn seed planting in Germany and in Indiana(USA), respectively. Direct intoxication of forager bees is likely related to consumption of contaminated nectar as well as to plant guttation of treated plants, which may cause direct kill of forager bees or a change of forager bee behavior, such as homing failure and disorientation. However, neonicotinoids may cause also intoxication inside the hive due to the transportation of contaminated pollen, which does not harm directly forager bees. However, this pattern has been less investigated.

To determine whether neonicotinoids may affect honeybee vitality and colony performance due to their transportation inside the hives we sampled bees and bee products (pollen loads, honey, wax, bee bread and royal jelly). Hives were located inan apple orchard of Trentino region (Italy), in which imidacloprid treatments are usually done after apple flowering to control aphid populations. Hives were placed few weeks before apple flowering and samples were taken before and after insecticide applications on April 18, 2012 and May 18, 2012, respectively. At the end of field test hives were transferred to a different location, with subsequent record of vitality and colony consistence parameters, every two weeks till winter. Results showed that imidacloprid was present in pollen loads even before treatments (~15 ppb) and increased strongly after treatments (~66 ppb). A similar result was found in wax and honey, whereas beebread did not show a significant change. Royal jelly showed a slight contamination of imidacloprid 2 months after treatments, equal to ~0.3 ppb. Our results suggest that imidacloprid may play a chronic detrimental effect on colony vitality, even several weeks after treatments.

Detection and Phylogenetic Analysis of Honey Bee Viruses in Asian Honey Bees and European Honey Bee in Northern Thailand

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Deformed wing virus (DWV), Sacbrood virus (SBV) and Black queen cell virus (BQCV) have been reported in European honey bee (*Apis mellifera*) in many countries around the world. The virus infections lead to the death of honey bee. These three viruses were presented in *A. mellifera* in Northern Thailand by using RT-PCR technique. In European honey bee colonies, DWV infection could be detected more frequently than SBV and BQCV especially, in Phrae province. However, only BQCV were found in all three Asian honey bee species (*Apis cerana*, *Apis dorsata* and *Apis florea*). The phylogenetic analyses of the three honey bee viruses in Northern Thailand were similar within groups. The viruses infected Asian honey bees were clearly separated from the isolates from *A. mellifera*. In addition, BQCV from all four honey bee species had closely related to the Hungarian BQCV isolate, previously reported in *A. mellifera*. BQCV in *A. cerana* was more closely related to BQCV isolate in *A. florea* than the isolates from *A. mellifera* and *A. dorsata*.

Optimization of *Varroa* treatment by formic acid application

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Formic acid is a widely used active ingredient to treat against Varroa destructor in honeybee colonies. It is a preferred form of treatment in Germany and is likewise used in other parts of Europe. Over the last few years, many different methods of treatment against Varroa using formic acid have become available on the market. However, not only are the methods of treatment varied but the amount of formic acid used can differ as well by anything from 60% to 85%.

An investigation by different German bee institutes, published in 2013, showed no significant differences in the effectiveness of the different treatment methods between using 60% or 85% formic acid. This result brought more clarity for beekeepers in applying the right concentration of active substance. The questions which are still not answered, however, are how beekeepers can distribute the acid in the hive in the best way to ensure maximum effect and how efficient the formic acid actually is at controlling the mites in the bee brood.

To deepen our understanding of this, we are carrying out an investigation into the diffusion of acid through the capped cells. First trials, launched in early spring 2013, involved sampling cell caps at several different time stages of bee brood. Initially, in order to understand how a cell cap is structured, we studied them under a scanning electron microscope (SEM). First images showed large holes in the structure of the cell caps which indicated that an exchange between hive air and cell air exists. Further laboratory trials showed formic acid diffusion thought the cell caps and a resulting alteration to an acidic ph-value of the air in the brood cells, as expected.

BEENET, a network for monitoring honey bee mortality and colony losses in italy

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The BeeNet monitoring network was established in September 2011, with an increase of the number of apiaries compared to the previous Apenet network (2009-2010). In 2011 there were 97 apiaries for a total of approximately one thousand beehives. In 2012 the monitoring network progressed up to 303 apiaries located in all the Italian regions with approx. 3,000 beehives. Each monitoring unit is composed of five apiaries with ten beehives each, managed by a referent person who is in charge to carry out visits in 4 different periods of the year: 1st, end of Winter; 2nd, Spring-Summer; 3rd, end of Summer-beginning of Autumn; 4th, before wintering. At each visit, environmental and beehive data are recorded, while at visit 1 and 3, samples of beehive matrices are collected (beebread and alive honey bees) to carry out chemical (pesticides), pathology (*Nosema*, virus and *Varroa*) and nutritional (pollen raw protein) analyses. In 2011 the total mortality was not correlated to any of the investigated parameters. However, the total mortality almost doubled in apiaries infected by *Nosema* compared to the negative ones. Furthermore, *Varroa* infection is directly correlated to ABPV, while Winter mortality is negatively correlated to the percentage of pollen raw protein. According to the data obtained from the first six months of monitoring in 2012, the infection by *N. ceranae* is between low and medium; DWV is present in 95.1% of the samples and in 20% of cases the concentration is above 10 million viral copies per bee. ABPV and CBPV are also present, with a prevalence of 50 and 70% respectively, but the number of samples with a viral load above 10 million viral copies account only for 1 and 3% respectively. Beebread collected from beehives located in southern Italy showed a higher raw protein content; however, the percentage of beebread contaminated by active substances used in *Varroa* control is higher than in beebread collected in northern Italy. Overall 50.4% of pollen samples analyzed was positive to at least one active substance. Moreover, a bee emergency service team has been established who is in charge of field intervention, samples and data collection, and epidemiological investigation in case of mortality report, in collaboration also with health authorities.

Genetic Diversity Based on Morphometric Analysis of Honey Bee Population of Khairpur in a Global Context

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Honey bees are described by genetic and morphological diversity. This study addresses partitioning the morphometric variance within and between honey bee populations and find the relationship between different honey bee populations. For this purpose, morphometric data of honey bee from five other studies were taken and compared with that obtained from district Khairpur. A total of fourteen characters of one hundred worker bees from Khairpur were studied and only common characters from Khairpur and world datasets were used for comparative study which were fore wing length, tibia, femur and metatarsus lengths. The data was organized and analyzed by Multivariate Statistical Analysis, Mean, Standard deviation, Principle Component Analysis (PCA), Discriminant Function Analysis (DFA) and Cluster analysis (CA). Statistical results showed that significant differences were present in Khairpur honey bee populations and other honey bee data sets from Ilam, Khuzestan, Bushehr, Hormuzgan, Cambodia, N. India, S. India, Srilanka, Iran, Mayanmar, Nepal, Oman, Pakistan, Thailand and Vietnam. Principle component analysis placed all the honey bee groups in four separate morphoclusters. Dendarogram constructed from a cluster analysis for the samples previously defined formed three main groups.

Key words: Khairpur, Morphometric analysis, Multivariate analysis, Dendarogram.

SWOT Analysis of B^3 (Biomonitoring of Bees as Bioindicators)

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A SWOT analysis of the most important Strengths, Weaknesses, Opportunities and Threats of B3 (Biomonitoring of Bees as Bioindicators) will be explained in order to this methodology can be approved as a skill to the certification of Environmental Quality or Environmental Risk Assessment. Different actions will be proposed about this objective and participants in Coloss Conference will be able to contribute with their suggestions.

The uncontrolled use of neonicotinoids is a threat to world beekeeping

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Advantages inherent to neonicotinoids as a means of plant protection do not guarantee their complete safety of the insect pollinators. Condition for collateral effects of neonicotinoids on honey bees is the fact that the use of this group of preparations against pests phytophages coincides with the period of vigorous activity of honey bees in agricultural lands. When exposed to the honey bee some neonicotinoids in sublethal doses or concentrations, a number of negative effects which causes the decrease in the number of bees in the colonies has been identified. For the development of rational measures providing safe use of neonicotinoids in the protection of plants as well as the honeybee at pollination of entomophilous cultures and honey collection the sufficiently complete information on the nature and characteristics of their toxic effects on insects of this species are needed.

The objects of study were working individuals Apis melliffera. As neurotoxicant we used the preparative form of imidacloprid (Tanrek) at sublethal and median lethal concentrations. Treatment of insects was carried per os, imidacloprid was diluted in honey syrup. A day and three days later we evaluated the state of intestine, fat body and morpho-functional structure of hemocyte and determined enzyme activity of phenoloxidase and antioxidant defense systems in the hemolymph and gut of bees.

Consequences of the imidacloprid action were pathological changes of intestine. Length and width of the hindgut were increased. Hyperemia and decreased elasticity of walls, the high sensitivity to mechanical damage, and inhibition functions of stinger were observed. Imidacloprid toxic effect was accompanied with pathological changes of adipocytes: deformation, vacuolization, disruption of membranes and lysosomes, increased with the concentration of the toxicant. Sublethal intoxication with imidacloprid was accompanied by a moderate increase in phenoloxidase positive hemocytes. Imidacloprid effect at the medium lethal concentration caused significant reduction of the proportion of phenoloxidase positive hemocytes, probably due to reduction in the number of granulocytes subjected to lysis and aggregation. The insecticide action also caused an increase in proportion of peroxidase positive hemocytes. Cytochemical analysis results are confirmed by the data of spectrophotometric determining of the protective enzymes activity. In general, the observed pathological processes in honey bee intestine and hemolymph at the action of imidacloprid similar to those that occur during the development of intestinal infection in insects. The neurotoxic effect of imidacloprid even at sublethal concentration causes a disruption of the functioning of systems of honey bee individual immunity, significantly reducing the viability of insects under pathogenic load.

COLOSS questionnaire is good tool for monitoring honey bee losses

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Bosnia and Herzegovina have (five years active monitoring) results that were achieved with internationally proposed COLOSS questioner.

Data of total winter loses were as follows: 2008/2009:**10.23%**;, 2009/2010:**8.6%;,** 2010/2011:**13.7%;,** 2011/2012**: 20.23%,;** 2012/2013:**6.18%.**

Concerning that this way achieved data are only one that existing in Beekeeping sector in Bosnia and Herzegovina, we will try to establish future monitoring in much wider scale.

Disruption of *Varroa destructor* honeybee chemosensing, for future Varroa management

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Evaluation and characterization of colony losses in Israel, through beekeepers' surveys and regular seasonal monitoring of selected hives over the last four years, clearly indicated that Varroa and its associated viruses are the most significant causes of colony losses. Varroa management based for the last thirteen years on country wide treatment with strips of "CheckMite+" (Bayer), is no longer effective. Apparently, the reason is resistance to the Coumaphos developed by the Varroa, while the resistance to Fluvalinate also exists in some of the areas. In attempt to develop a new approach for Varroa control we are investigating the possibility to cope with the mite, by interfering with its sensing the honey bee. We hypothesize that disrupting the natural preference of the mite for the nurse bee will sabotage its successful colonization of the brood cells and thus the reproduction.

In laboratory experiments we examined the possibility to disrupt the Varroa – honeybee interaction by targeting the mite's olfactory system. In particular we examined the effect of synthetic chemicals originally develop to disrupt pheromone detection by moths. We tested the effect of these compounds on the Varroa chemosensory organ by electroantenography (EAG) and on its behavior in a choice bioassay.

We found that in the presence of some of the tested compounds but not the others, EAG response of Varroa chemosensory organ to honey bee headspace volatiles, significantly decreased. Furthermore, disruption of the Varroa volatile detection was accompanied by a loss of Varroa's significant preference for a nurse over a forager bee. Although still far from practical application, this approach may open new venues for future Varroa management.

Nosema ceranae has been present in Brazil for more than three decades

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As far as the beginning of the 90's it was believed that the only microsporidian related to bees of the genus *Apis* was *Nosema apis*. A second specie, *N. ceranae*, was observed only in 1996 in Asian honey bees and it is postulated that it was probably transmitted from *Apis cerana* to *Apis mellifera*. Currently, the specie *N. ceranae* is present on five continents and is often related to phenomena of collapsing honey bee colonies. Samples of drones collected in 1979, preserved in alcohol, originated from the UFRGS, in Porto Alegre, Brazil, were sent for analysis at the Honeybee Health Laboratory (LASA) of the APTA/SAA-SP. As stated by the University, the encoding of the sample referred to the collection formed by the late teacher of the Department of Plant Protection. Light microscopy analyses were performed in order to count spores. After DNA extraction, PCR-duplex were conducted. Negative and positive controls were used. All molecular analysis (triplicate) indicated that the drones were co-infected with *N. ceranae* and *N. apis*. Molecular analyses were repeated in another Federal Laboratory (Genetic Molecular Laboratory/EMBRAPA). PCR products were sequenced and matched to sequences reported in GenBank (Acc. No. JQ639316.1 and JQ639301.1). The venation pattern of the wings of these males was compared in the USP to the current population living in the same area and with drones collected in 1968 in Ribeirão Preto, SP, Brazil, from a location close to the release site of Africans swarms in 1956. The morphometric results indicated that the population collected in 1979 was different from the current living population, both of which are more differentiated when compared to the older population. Considering that the use of molecular tools for differentiating species of *Nosema* is relatively new, it is possible that less recent reports of infections presented infections of *N. ceranae* wrongly diagnosed as those of *N. apis*. Although our results show that *N. ceranae* has been affecting Africanized honeybees in Brazil for at least 34 years, the impact of this pathogen remain not clear.

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